STUDIES ON THE VENOUS PULSE. I.

A Study of the Diastolic Waves of the Venous Pulse, with Especial Reference to the Possibility of a Wave Due to the Contraction of the Venous Region of the Mammalian Heart.[1]

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Plates XX and XXI.

The following communication is based on the study of the venous pulse tracings from sixty normal men, venous pulse tracings obtained from dogs by the use of an intraauricular or intravenous sound, and finally the effects produced on the venous pulse waves of the dog by extrasystoles arising from stimulation of the venous or so-called sinus region of the heart.

Methods.—The venous pulse in man was recorded with the arterial pulse from the carotid artery, in some cases with the apex beat, by the usual technique. A Mackenzie transmitter was employed and was connected with a delicate recording tambour with compound lever. In the work on dogs, the auricular or venous pulse curves were obtained by the use of the venous sounds described and employed by Fredericq (1). In large dogs under morphia-ether anesthesia, about eight centimeters of the external jugular vein were dissected free and carefully cleaned. Four to six centimeters were then removed, everted over the large end of the sound and tied. The sound was then introduced into the vein at varying depths or directly into the right auricle and connected with a recording tambour. The arterial pulse from the carotid artery was recorded simultaneously on the record by means of a Hürthle manometer, and in most of the experiments the pressure changes in the left ventricle were recorded also by means of a ventricular sound connected with

[1] Received for publication February 21, 1910.

a second Hürthle manometer. In these experiments the thorax was not opened. In three experiments, extrasystoles were obtained by stimulation of the venous region of the heart and the auricles with single induction shocks. The technique used to record the venous pulse in these experiments was the same; the thorax was, however, opened under artificial respiration after preliminary ligation of the internal mammary arteries and the ventricular contractions recorded directly by air transmission, the tip of the ventricle being attached by a thread to a transmitting tambour which connected with a recording tambour writing on the record. A stimulating electrode was applied to the venous region of the heart or to the auricle and the incidence of stimulation with single induction shocks recorded by means of a magneto pen in the primary circuit. The air used for artificial respiration in the experiments with open chest was warmed, and in order to prevent the very rapid heart rate which is usually present in the dog under these circumstances, following the suggestion of Henderson (2), the dead space was increased to one hundred centimeters or more.

WAVES ON THE VENOUS PULSE IN MAN IN THE INTERVAL BETWEEN THE V AND A WAVES.

Historical.—In recent years a number of observers have noted a wave on the venous pulse occurring in diastole in the interval between the *v* wave and the *a* wave of the following cardiac cycle. A division of the *v* wave into two parts, a late systolic and an early diastolic portion, the division corresponding in time to the closure of the aortic semilunar valves as shown by the dicrotic notch on the carotid tracing, has been noted by Bard (3), Mackenzie (4), Wenckebach (5) and others in man, and by Fredericq (1) and Hering (6) in venous pulse curves obtained from dogs. The gradual rise of venous pressure following the *v* wave and continuing up to the next auricular wave, especially evident in slow heart rate, has been also noted, and is designated in the German literature by the letter *s* ("Stauungswelle") (Rihl (7)). The first description of separate and distinct positive wave in the late diastolic period, or the period of diastasis of the heart (Henderson (8)), was made about the same time by Fredericq (1) in the dog and by Hirschfelder

(9) in man. The tracings published by Thecpold (10) in the same year show a similar wave during vagus stimulation. Fredericq found that the wave observed by him occurred only with slow heart rate but suggested no explanation. Hirschfelder states that while a positive wave on the venous pulse between the *v* and the *a* wave of the next cardiac cycle is only occasionally present, it is probably the expression of a normal occurrence in the heart during this period. He has designated this wave by the letter *h,* and has offered as a possible explanation the floating together of the tricuspid valves, which, according to the view of Henderson (8), occurs following the period of most active ventricular filling. The same wave was described by Gibson (11) a few months later. Gibson designated this wave on his tracings by the letter *b* and offered a similar explanation to the one advanced by Hirschfelder. Gibson noted the presence of a third heart sound in two of the five cases in which this wave was present and advanced the opinion that the wave and the sound are expressions of the same occurrence in the heart. Thayer (12) noted the relation of the diastolic venous pulse wave to the third heart sound, the protodiastolic elevation of the cardiogram and the shoulder of the ascending limb of the volume curve of the ventricle. He regarded the evidence to be in favor of the view that the sound and wave were produced by a sudden tension of the auriculo-ventricular valves occurring at the end of the first and most rapid phase of cardiac filling.

Results from the Study of the Venous Pulse Tracings Obtained from Normal Men.—The occurrence of waves on the venous pulse curve from normal man between the *v* and *a* waves depends apparently upon two factors, the heart rate and the delicacy of the recording mechanism. The *a, c* and *v* waves are evidently the predominating waves on the venous pulse, and if the heart rate is faster than a certain maximum, any additional waves which may be present are obscured by the *v* wave and the following *a* wave. It is the interval between the *v* and *a* waves which especially varies with changes in cardiac rate. An increase of cardiac rate is associated with an approach and partial fusion of the *v* and *a* waves, a decrease in rate, on the other hand, with a separation of these two waves. In the great majority of cases in normal man, if the heart rate is not too

fast and the recording apparatus sufficiently delicate, one or two waves are apparent on the venous pulse tracing in this interval. A heart rate of seventy will in nearly all cases show one of these waves. This wave, from its position and character, is evidently the wave designated by Hirschfelder as *h* and by Gibson as *b*. A somewhat slower heart rate will in many cases, but not invariably, show the addition of a well-developed wave between the *h* and *a* waves. This wave is designated in the tracings by *x*. Plate XX, Fig. 1, shows this wave in the jugular tracing obtained from a normal individual with a heart rate of fifty-eight beats to the minute.

Increase of the heart rate by exercise leads to a disappearance of both the *h* and *x* waves, the shortened diastole being manifested by an approach of the *v* and *a* waves. Plate XX, Fig. 2, shows a tracing from the same individual from whom the tracing shown in Fig. 1 was obtained, after exercise with a pulse rate of ninety-eight beats to the minute. The importance of the length of the cardiac cycle in the development of these waves is shown in the three cardiac cycles reproduced in Plate XX, Fig. 3. The first of these, *A*, has a total length of 0.95 second. It shows a well developed *h* wave but no *x* wave. *B*, the third subsequent cardiac cycle, has a length of one second, and it may be seen that the increase in length is mainly the result of an increase in the interval between the beginning of the *h* wave and the beginning of the following *a* wave. The *h* wave in this cycle shows indications of a division into *h* and *x* waves, and in the fourth subsequent cardiac cycle, shown in *C*, there is distinct *x* wave with a longer *h-a* interval than in the preceding cycles. The same conditions leading to the development of an *x* wave are evident in the two cardiac cycles shown in Plate XX, Fig. 4. The first of these, *A*, is a few hundredths of a second shorter than the fourth subsequent cycle from the same individual shown in *B*. In *B*, the *h-a* interval is greater, and an *x* wave, scarcely evident in *A*, is shown distinctly separated from the *h* wave in *B*.

The *x* wave would seem, therefore, to be a normal occurrence in the cardiac cycle, and its presence to be conditioned, in part at least, upon the presence of a sufficiently long period of diastasis to pre-

vent its obliteration by the *h* and *a* waves. The *x* wave is not, however, invariably present when this condition is fulfilled. This may mean the absence under certain conditions of those occurrences which produce it, or its failure to be recorded upon the tracing due to lack of sufficient delicacy in the recording mechanism. The *h* wave, however, according to the experience of the present writer, is always present when the cardiac rate is sufficiently slow to prevent its confusion with the *v* and *a* waves.

THE H WAVE IN THE VENOUS PULSE TRACINGS FROM DOGS.

The presence of an *h* wave in venous pulse curves from dogs seems to be conditioned by the same factors as condition its appearance in man, namely, a slow heart rate and delicate recording apparatus. When the *v* wave approaches close to the *a* wave of the next cycle, due to increase in pulse rate, the *h* wave disappears or is obscured by the *a* wave. Plate XXI, Fig. 5, is a tracing obtained from a dog with unopened thorax. The uppermost curve is the venous pulse for the right auricle. The second curve from the top is the left intraventricular pressure, the third curve is the arterial pressure from the left common carotid. The slowing of the heart that occurs toward the middle of this tracing is the result of stimulation of the vagus by a weak faradic current. The *h* wave, at first not present, becomes evident as the heart rate is slowed, and disappears again as the rate increases.

The *h* wave, therefore, is a normal wave in man and the dog. Its presence on a venous pulse tracing depends upon a sufficiently slow cardiac rate to prevent interference by the following auricular contraction. It is purely a diastolic wave and lengthening of the cardiac cycle is mainly manifest by an increase in the distance between the *h* and *a* waves.

An additional wave on the venous pulse of the dog between the *h* and *a* waves has not been observed in these experiments, though in a number of cases the cardiac rate has been sufficiently slow to allow it to be recorded, if present.

CONSIDERATION OF THE POSSIBILITY OF THE X WAVE REPRESENTING A CONTRACTION OF THE VENOUS OR SINUS REGION OF THE HEART AND THE BEHAVIOR OF THIS REGION IN EXTRASYSTOLES.

Evidence from the Literature for the Presence of Contraction in the Venous Region of the Heart and the Occurrence of the Condition of Sino-auricular Block.—A number of observers have noted definite rhythmical contractions in the region of the mouth of the great veins in hearts *in situ,* dying from asphyxia, and in excised hearts when the perfusion is interrupted. Each venous contraction may be followed by an auricular systole, or there may be evidence of a partial veno-auricular block, shown by the auricles contracting to every second, third or fourth beat of the vein. This latter condition has been described in partially asphyxiated hearts *in situ* by Hering (13) and by Hirschfelder and Eyster (14), and in excised hearts by Hering (15) and by Erlanger and Blackman (16). Wenckebach (17), Hirschfelder (18), Hewlett (19) and Rihl (20) have reported clinical cases in which the venous pulse showed an occasional interval between two auricular beats equal to two normal auricular cycles. The assumption in these cases has been that in the interval there was a beat of the vein or sinus region of the heart, and in consequence of the presence of a partial veno-auricular or sino-auricular block, the impulse did not reach the auricle. The evidence for block in these cases is hence purely indirect; no wave which could be referred to a contraction of the sinus region was present. Erlanger and Blackman (16) have produced a similar condition, as shown by the auricular rhythm, in the excised rabbit's heart by placing the region of the great veins under slight torsion. No one, so far as I am aware, has observed contraction of the venous region of the heart under approximately normal conditions, and while it seems unquestionable that this region may manifest contractility under the influence of asphyxia and perhaps other abnormal conditions, the question as to whether it normally shows contractility is an open one.

Does the x Wave Represent a Contraction of the Venous Region of the Heart?—In many cases in man, the *x* wave precedes the *a* wave by a period of time no greater than the *a-c* interval. Under these circumstances, its origin might be explained upon a contrac-

tion of the venous or sinus region of the heart. It has been shown by Hirschfelder and Eyster (14) that in the dog in the case of extrasystoles arising from stimulation of the venous region of the heart, the impulse is transmitted to the auricle with a period of veno-auricular or sino-auricular conduction which is somewhat less than the time consumed in auriculo-ventricular conduction. The following fact is, however, strongly against the interpretation of the *x* wave as a sinus wave, and would tend to show that it is a diastolic wave conditioned by some factor occurring toward the end of the period of diastasis. If the length of a number of cardiac cycles varies slightly in a tracing which shows a well developed *x* wave, it may be seen that it is largely the *x-a* interval which is affected in this variation. In other words, a lengthening of the cycle is manifested by an increase in the separation of the *x* and *a* waves; the difference in length of any two cycles is manifested by an approach or divergence of the *x* wave and the *a* wave of the next cardiac cycle. Plate XXI, Fig. 6, shows three cardiac cycles of slightly different length. The pulse rate is sixty to the minute. The first cycle shown in this tracing is the shortest; it shows an *h* wave but no *x* wave. The *x* wave is clearly evident in the next cycle. The third and longest cycle shows a separation of the *x* wave from the *a* wave, which is equivalent to, so far as can be determined from measurement, the difference in length between the second and third cycles. The *x-a* interval is represented by the distance between the scratch lines 3 and 4. The difference in length between the second and third cardiac cycles is represented by the distance between the scratch marks numbered 5 and 2. Plate XXI, Fig. 7, shows a tracing from the same subject when the heart rate was forty-three to the minute. The *x* wave is separated from the *a* wave by a much longer interval than in Fig. 6, and there is a considerable period before the *a* wave in which the venous pulse is quiescent and shows no wave. It would seem from the above considerations that the *x* wave is a true diastolic wave and referable to some change in the flow of blood occurring during this part of the cardiac cycle.

The Venous Pulse in Extrasystoles of the Heart Arising from Stimulation of the Venous Region.—The fact that an extrasystole of the mammalian heart may be produced by stimulation of the

great veins just beyond their place of entrance into the right auricle has been shown by a number of observers. Whether the region stimulated actually contracts as a result of this stimulus or simply develops and transmits the impulse to the auricle has never been determined. Hirschfelder and Eyster (14) showed in the dog that stimulation of the superior vena cava near the auricle produced an extrasystole of the heart with an interval between the incidence of the stimulus and the contraction of the auricle which was 0.02 to 0.04 of a second longer than the latent period of the auricle to stimuli applied directly to it. There was thus demonstrated a veno-auricular conduction period which varied between 0.02 and 0.04 of a second. This period of time was evidently consumed by the impulse, set up in the vein as a result of the stimulus in passing from the vein to the auricle. That the conduction is a physiological one and not due to spread of electrical current, was indicated, in the first place, by the time consumed in the conduction; secondly, by the fact that it occurred from mechanical as well as electrical stimulation of the vein; and finally the passage of the impulse was prevented if the vein close to the auricle was crushed in a clamp, continuity being preserved so as not to interfere with electrical conductivity. These results led to the conclusion "that there is a physiological as well as anatomical path connecting the superior vena cava with the two auricles, which conducts an impulse arising in the former to the latter according to physiological laws."

The question as to whether or not the region of the mouth of the great veins contracts when stimulated seemed to offer the best hope of solution by stimulation of this region during the latter portion of diastole and noting the effect on the venous pulse tracing. If the cardiac rhythm is sufficiently slow to give a distinct interval between the *h* and *a* waves, during which time the venous pulse in the dog is quiescent, stimulation of the venous region in this period should give, if contraction of the vein results, evidence of the contraction on the venous pulse tracing. Any venous contraction resulting at any other time in the cardiac cycle might give no evidence on the venous pulse curve because of the presence of other and stronger waves resulting from other cause, namely, the *a, c* and *v* waves. If the venous region is stimulated during the period de-

scribed above, we might expect one of two results. If the vein contracts, a wave should occur on the venous pulse tracing, and after a short period, representing the *v-a* conduction period, be followed by the auricular wave. On the other hand, if the venous region does not contract as a result of the stimulation, but simply develops and transmits the impulse to the auricles, no wave should occur on the venous pulse until the *a* wave and this should be followed by the ventricular contraction after the regular *a-c* interval.

In the numerous extrasystoles from stimulation of the venous region of the heart obtained in these experiments, the latter condition described above was the one invariably present. No wave which could be referred to a contraction of the venous region occurred in any case. An example is given in Plate XXI, Fig. 8. There are shown in this tracing three extrasystoles from stimulation of the venous region of the heart near the auricle. In the first two extrasystoles only does the incidence of stimulation fall in the period of quiescence of the venous pulse between the *h* and *a* waves.

Interpretation of Fig. 8.—The venous sound in this case was at a point just above the entrance of the great veins into the auricle. The *a–c* interval in the first cardiac cycle before the first extrasystole is 0.084 of a second. The length of the complete cardiac cycle in this case is 0.41 second. In the first extrasystole, which results from a stimulus falling in 0.2 second after the beginning of the previous ventricular contraction, there is no wave on the venous pulse until 0.094 second later, when a wave occurs, evidently the *a* wave, with an *a–c* interval of 0.073 second. The next regular *a–c* interval is 0.084 second. 0.158 second after the beginning of the next regular systole, the venous region is stimulated. There is no wave on the venous pulse until 0.075 second later, when the *a* wave occurs, with an *a–c* interval of 0.068 second.

In the third extrasystole, the stimulus falls 0.080 second after the beginning of the preceding ventricular contraction and about 0.02 second after the beginning of the *v* wave, the result of the previous ventricular systole. The *v* wave retains the usual shape and duration shown in the previous cardiac cycles, and 0.068 second after the incidence of the stimulus, the auricle contracts as shown by the *a* wave on the venous pulse; 0.089 second later the ventricle contracts.

The time from the incidence of stimulus to contraction of the auricle represents the *v–a* interval plus the latent period of the auricle. The *v–a* interval in the dog varies between 0.02 and 0.04 second. The remainder of the time consumed represents the latent period of the auricle, which, except in early extrasystoles, varies between 0.025 and 0.05 second (Hirschfelder and Eyster (14)).

The above relations may be expressed in tabular form as follows:

Cardiac cycle.	Time from incidence of stimulus to contraction of auricle.	*a–c* interval.	Occurrence of stimulus after beginning of preceding ventricular systole.
Normal cycle		0.084	
First extrasystole	0.094	0.073	0.200
Normal cycle		0.084	
Second extrasystole	0.075	0.068	0.158
Third extrasystole	0.068	0.089	0.080

Plate XXI, Fig. 9, shows an extrasystole arising from stimulation of the right auricle. After a latent period much shorter than in the extrasystoles shown in Fig. 8, the auricle contracts, and this is followed, after the usual *a-c* interval, by contraction of the ventricle.

It is evident, therefore, that while a stimulus applied to the venous region of the dogs heart will cause an extrasystole, with the presence of a definite period of veno-auricular conduction, that no contraction of the venous region occurs which is sufficiently pronounced to influence the venous pulse. It would seem probable that in the dog under approximately normal conditions, the venous region of the heart, although capable of developing an impulse from a stimulus and transmitting the impulse to the auricle, does not itself contract. As is well known, under certain abnormal conditions, namely, asphyxia and interruption of the circulation, this region may develop contractility.

SUMMARY AND CONCLUSIONS.

A wave is described occurring in the venous pulse of man late in the diastolic period. This wave is a diastolic wave, since in an increase in length of the cardiac cycle it follows the *h* wave at its regular interval and becomes separated from the *a* wave of the next cardiac cycle. There is no wave on the venous pulse in man which can be referred to a contraction of the region of the great veins or sinus region of the heart.

The *h* wave is a normal occurrence in the venous pulse curve from man and the dog. It occurs, according to the experience in this work, in all normal individuals in which the heart rate is sufficiently slow to prevent its obliteration by the *a* wave. The *x* wave, while not infrequent, is not always present in man even when the cardiac cycle is of sufficient length to prevent its obliteration by the *a* wave. This wave has not been observed in the dog.

There is no wave on the venous pulse curve of the dog that can be referred to a contraction of the sinus region of the heart. Extrasystoles from stimulation of the venous region of the dog's heart produce no contraction of this region sufficiently pronounced to be recorded on a venous pulse tracing. After an interval of from 0.07 to 0.1 second, representing the interval of veno-auricular conduction plus the latent period of the auricle, the auricle contracts. Extrasystoles from stimulation of the right auricle show, after an interval following the stimulus much shorter than that noted above and representing the latent period of the auricle, an *a* wave followed by ventricular systole after the usual *a-c* interval. It would seem probable that the sinus region of the heart, while capable of irritability, conductivity and rhythmicity (Erlanger and Blackman (16)), does not normally manifest contractility.

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EXPLANATION OF PLATES.

PLATE XX.

FIG. 1. Venous tracing from a normal man. Top line is jugular tracing; middle line, carotid pulse; bottom line, time in .2 second. Pulse rate 58 per minute. Record should be read from left to right.

FIG. 2. Venous tracing from the same individual as Fig. 1, shortly after exercise with a pulse rate of 98 per minute. Upper line is carotid; lower, jugular. Record should be read from left to right.

FIG. 3. Showing three cardiac cycles from a normal man. Upper line is jugular; lower, carotid pulse. For description of record see text.

FIG. 4. Showing two cardiac cycles from a normal man. Top line, jugular; middle, carotid pulse; bottom line, time in .2 second. For description of record see text.

PLATE XXI.

FIG. 5. Venous pulse from a dog during light Faradic stimulation of the peripheral end of the right vagus. Top line is tracing from sound in right auricle; second line from top, record of a Hürthle manometer connected with sound in left ventricle. The straight line at lower portion of the latter tracing is the line of zero pressure for the manometer. The third line from the top is the carotid pressure recorded by another Hürthle manometer, the line of zero pressure of which is the bottom line. The time record between these two is in intervals of .2 second. The record is to be read from left to right.

FIG. 6. Venous tracing from a normal man. Top line, carotid; middle line, jugular tracing. The bottom line records the time in intervals of .2 second. Pulse rate 60 per minute. For description of numbers see text. Record is to be read from left to right.

FIG. 7. Venous tracing from same individual as Fig. 6. Pulse rate 43 per minute.

FIG. 8. Venous tracing from dog with open thorax, showing three extrasystoles from stimulation of the venous region of the heart with single induction shocks. The top line records the venous tracing. The second line from the top records the contraction of the ventricles, the down stroke representing systole. The third line from the top records the time in intervals of .2 second. The bottom line marks the incidence of stimulation. For interpretation of record see text. Record is to be read from left to right.

FIG. 9. Tracing obtained from a dog under conditions similar to that shown in Fig. 8. The extrasystole, however, arises from stimulation of the right auricle. For interpretation of record see text. Record is to be read from left to right.

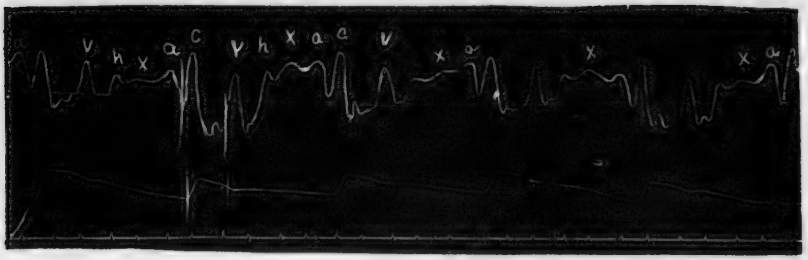


FIG. 1.

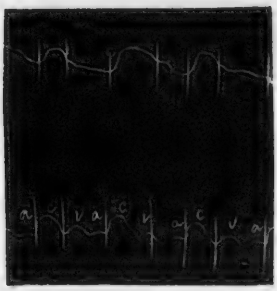
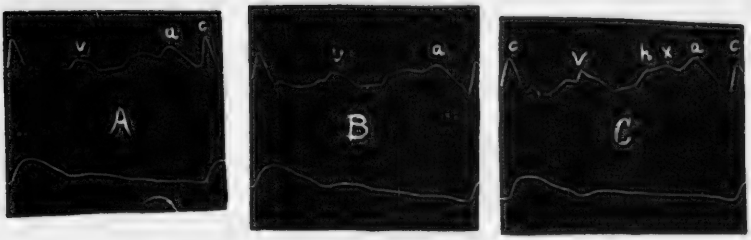


FIG 2.



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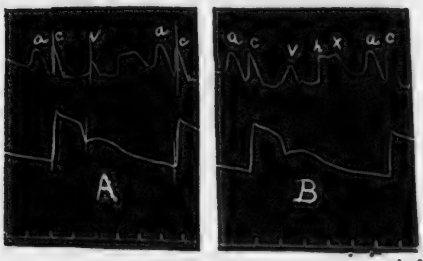

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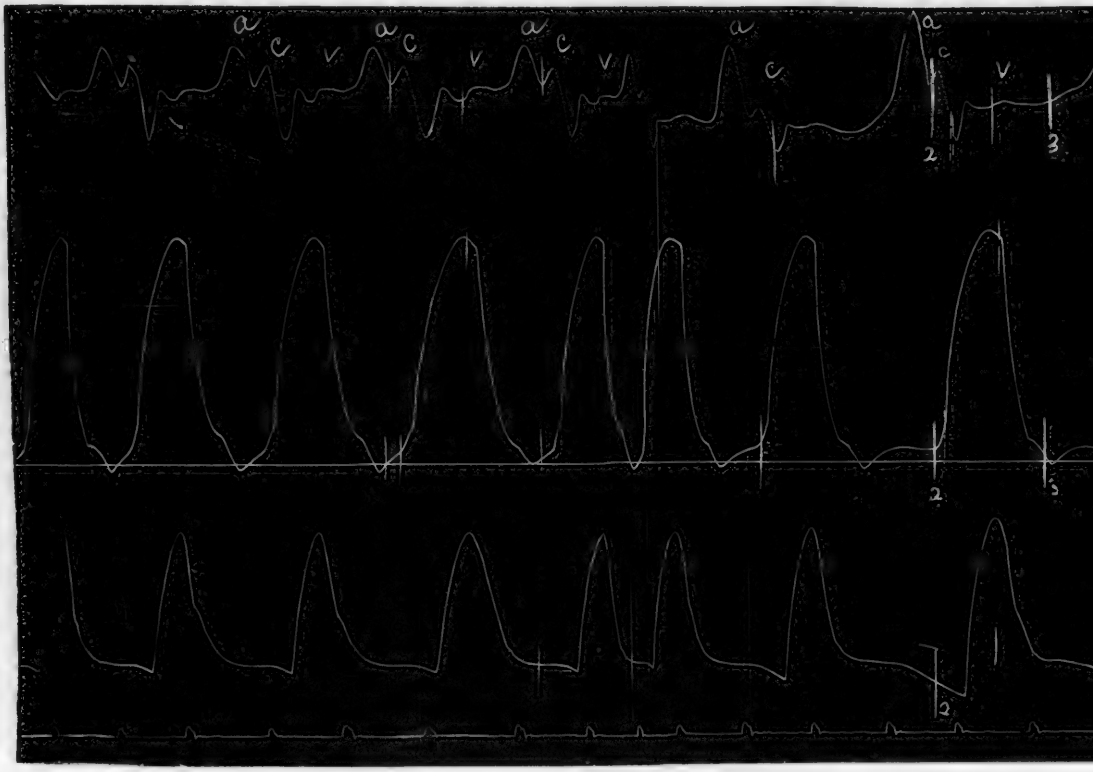


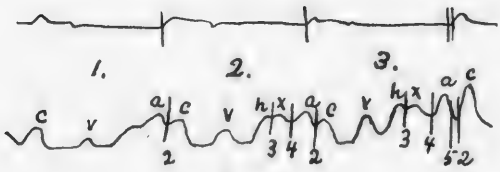


FIG. 6.

FIG. 7.

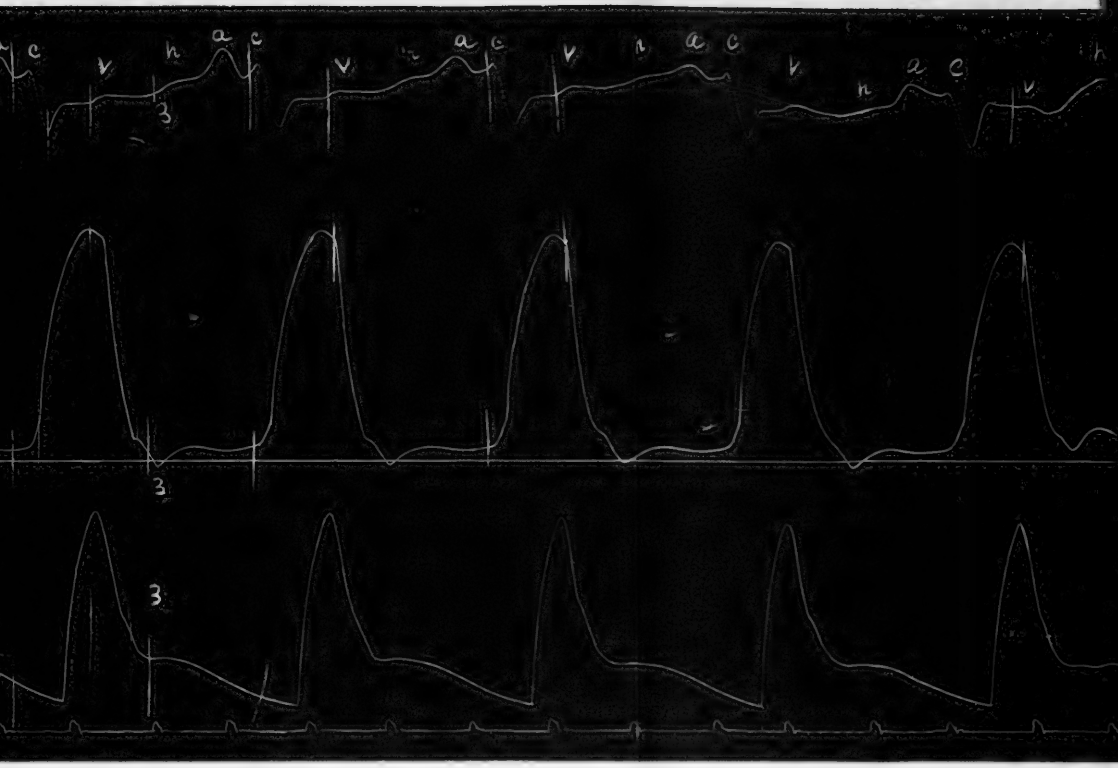


FIG. 5.

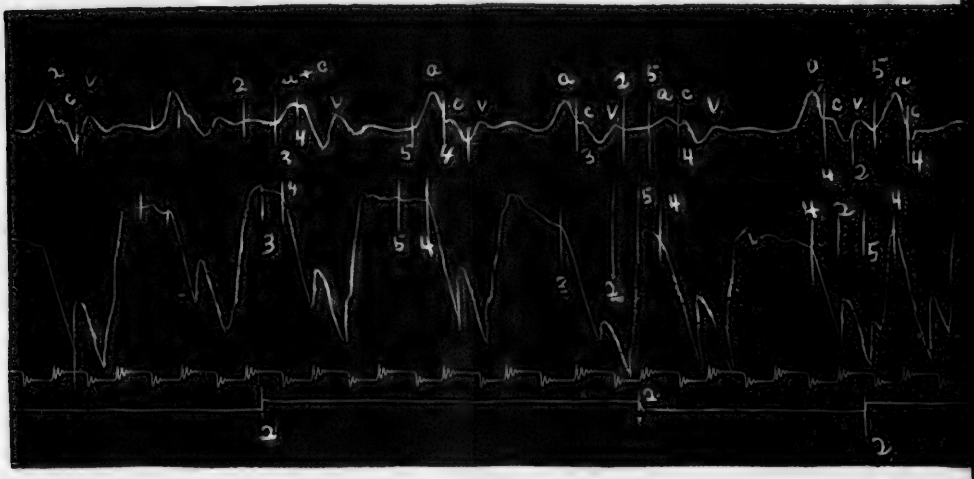
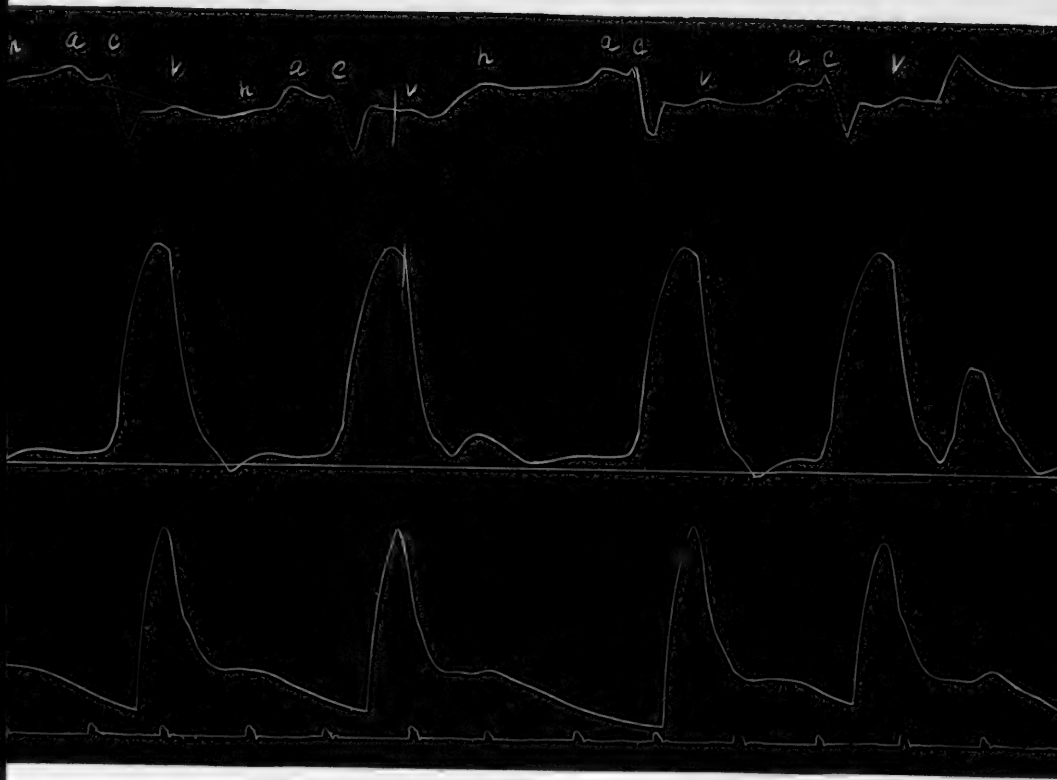


FIG. 8.



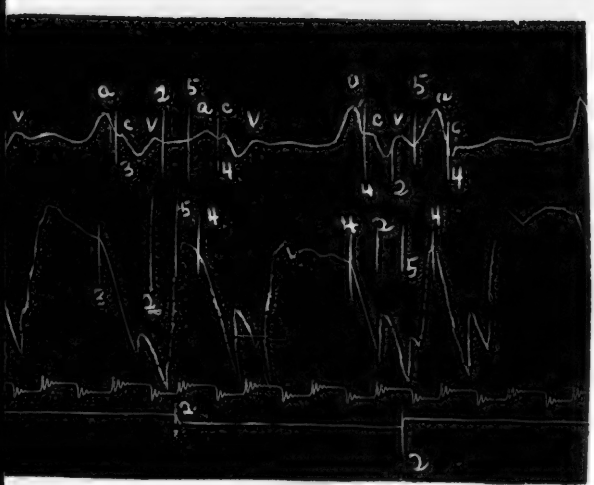
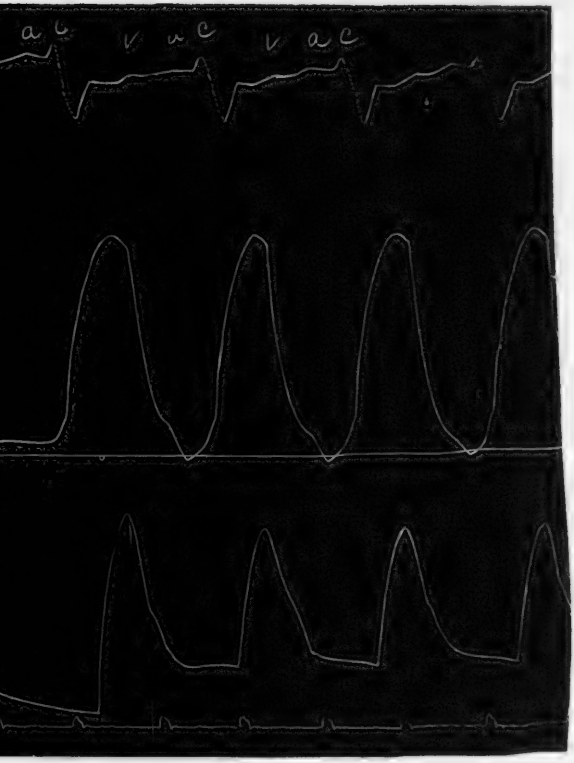


FIG. 8.



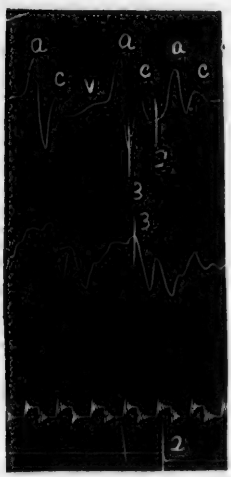


FIG. 9.

SURVIVAL OF ENGRAFTED TISSUES.

I. (A) Ovaries and (B) Testicles.[1]

By C. C. GUTHRIE.

(From the Physiological Laboratories of Washington and Pittsburgh Universities.)

Plates XXII and XXIII.

A great deal has been written on the subject of survival of engrafted tissues, and, as might be expected, many conflicting results have been reported. It is not our present purpose to review extensively the literature,[2] but rather to present some additional observations. Reference will be made to the work of other investigators in so far as may seem necessary in acknowledging priority, or in discussing results.

Methods.—We have employed two methods: (*a*) simple transplantation, that is, transplanting without anastomosis of blood vessels; and (*b*) transplantation with anastomosis of blood vessels. Blood vessel grafts for the purpose of restoring the continuity of blood vessels occupy a peculiar position and will be dealt with in the third paper of this series.

A. *Simple Transplanting or Engrafting.*—By this method tissues are removed and replaced, or are placed in a new situation in the animal from which they are removed, under the skin, into the peritoneal or other cavity, into organs, such as the liver or spleen, into muscle, the cavity of bones, etc. Such an operation is termed an auto- or homo-transplantation or graft. If the tissue be placed into

[1] Received for publication January 17, 1910. The second paper of this series entitled "II. (A) Thyroid (including parathyroid) and (B) Kidney (including adrenal)" appeared in *The Journal of the American Medical Association*, 1910, liv, 831.

[2] A condensed abstract of the literature is given with a paper entitled "On Graft Hybrids," read at the annual meeting of the American Breeders' Association, Omaha, December, 1909, and to be published in the proceedings of the Association.

another individual of the same species, iso- is the prefix customarily employed, while if the animal supplying the tissue be of a different species, hetero- is the prefix used. The animal supplying the tissues is called the donor, while the one receiving them is spoken of as the donee or host. In the case of the ovaries (or testicles), if offspring are produced from the transplanted tissue, and this has been realized in the case of the ovaries,[3] the relation of the parent bearing such tissue to the offspring has been indicated by the word foster, *e. g.*, foster-mother. Objection may be raised to these terms, but such a point is of etymological rather than of scientific interest and for the present at least we need not further concern ourselves with the matter of terminology.

The simplicity of the technique, of course, varies not only with the nature of the tissue employed and the place into which it is engrafted (a skin-graft is an operation of much less magnitude than the engrafting of the ovaries from one animal into another) but also with the animals employed. For example, fowls being more highly resistant to infection and shock than mammals, correspondingly less elaborate operative precautions are necessary when they are used.

When tissues are to be removed and placed in another animal, or if they are to be exchanged between two animals, both animals are operated upon as nearly as possible at the same time and the tissues when removed are immediately transferred to the host, the time of complete isolation and exposure being not more than a very few seconds. The tissues are sometimes secured in place with fine ligatures, while at other times they are thrust into cavities or pockets made for their reception, such cavities being closed with a stitch.

B. *Transplanting or Engrafting with Anastomosis of Blood Vessels.*—An illustrated and detailed account of the technique employed in this laboratory will be published later. It is superfluous to add that *small masses* and tissues from *young* animals give the best results. The nomenclature used for transplantations with anastomosis of the blood vessels is the same as for simple transplantations. The essential difference in technique is that in connection with union of the blood vessels.[4] By this method, larger masses of

[3] *Jour. of Exper. Zool.*, 1908, v, 563–576; *Science*, N. S., 1909, xxx, 724–5.
[4] *Jour. of the American Medical Assn.*, 1908, li, 1658.

tissues are employed than in the simpler operation. Also for obvious reasons (size of blood vessels, etc.) the more complex operation has not been performed on very small animals.

(A) OVARIES

A. *Simple Grafts.*—The most complete results have been obtained with ovaries in fowls. In auto-intra-abdominal as well as in subcutaneous transplantations, excellent morphological results have been obtained (Plate XXII, Fig. 1). In iso-intra-abdominal ovarian grafts between fowls and between guinea pigs, functional activity, in addition to anatomical structure, has been completely preserved (Plate XXII, Figs. 2, 3 and 4).[5]

Evidence of the complete functioning of such transplanted ovaries is afforded by the altered characters in the offspring. The ovary of a pure bred white hen was removed and in its place an ovary from a pure bred black hen was engrafted. Subsequently, the white hen laid eggs and on being mated to a pure white cock, such eggs were incubated and from them were hatched white and black chicks (Plate XXIII, Fig. 6).[6] As yet there is no evidence that simple mutilation of the ovary is a factor in these results.

To challenge this evidence of functioning seriously, it would be necessary to show that the eggs obtained from the hens into which ovaries had been transplanted were not from such transplanted tissue, but tissue originally belonging to the hen. Three possible explanations of the presence of such tissue might be advanced: (1) incomplete removal of the hen's ovarian tissue; (2) accidental intra-abdominal auto-grafting, that is, lack of complete removal of the ovarian tissue after separation from its attachments; (3) development of new ovarian tissue, perhaps from the peritoneum.

That the first possibility may actually happen occasionally is shown by Chicken 29. A hardly visible mass of ovarian tissue was no doubt overlooked at the time of the operation, owing to its relatively inaccessible position. Its relations with surrounding

[5] For result on offspring of fowls see *Jour. of Exper. Zool.*, 1908, v, 563; on guinea pigs, *Science*, N. S., 1909, xxx, 724–5.

[6] For further discussion see paper entitled "On Graft Hybrids," *American Breeders' Association, loc. cit.*

structures strongly indicate that it is tissue that escaped removal. A large ovarian mass separated completely from the small mass by a glistening peritoneal surface no doubt represents the engrafted tissue. Evidences in favor of this view are its abnormal connections and relations and its great size and position. For in no case has a normal ovary been observed with similar connections and relationships; nor, owing to its large size and to its easily observed position, can it be seriously advanced that it is tissue that escaped removal at the time of the operation. Also, many of the follicles of this mass are many times larger than the largest follicles in the small mass which, as already stated, probably represents tissue that escaped removal.

As illustrative of the second possibility which has been mentioned is a small isolated follicle described above, occurring midway between the two ovarian masses. It has apparently simply adhered to the peritoneum and no doubt is an auto-graft. The ovary was broken up more or less in removal, and this follicle was lost. Such isolated tissues have always been carefully searched for, but in none of the few instances in which they have been found, have they remotely approached in size a mature follicle. Indeed, the one under discussion, though not over one millimeter in diameter, is as large as any that have been observed. This seeming lack of development may be associated with their distance from large blood vessels, and hence deficiency in blood supply, those observed having been adherent to the peritoneum at some distance from the dorsal blood vessels (compare p. 276).

As to the third possibility, the development of normally functioning ovarian tissue from soma cells, our results are emphatically against the view. For example, in no case have hens from which the ovaries have been removed laid eggs subsequently.

Again in the single case in which degeneration of an ovary engrafted into a fowl has been observed no eggs were laid.[7] Furthermore, all control hens have produced eggs. A minute post-mortem examination of Chicken 35, in which degeneration of the engrafted ovary occurred, did not reveal any vestige of normal ovarian tissue, nor abnormality of any kind save the degenerated engrafted mass.

[7] The host was a white Leghorn pullet; the donor, a buff Cochin bantam.

We may also take into account the evidence furnished by castration or spaying of various animals. As is well known, removal of the ovaries from a young female is followed by a subnormal development of female characters, including sterility. The general effect on development may be so strong that on reaching adult age the unsexed animal may present qualities as strongly masculine as feminine. No such result was observed in hens into which ovaries had been engrafted after removal of their own ovaries, except in the case of degeneration of the engrafted tissue (Chicken 35). The results in this case were exceedingly pronounced. The pullet rapidly acquired not only the outward anatomical features of a cock—cock's comb, wattles, long hackle and tail feathers, rapidly developing spurs (Plate XXII, Fig. 5), carriage, etc.—but the behavior as well was that of a male; it exhibited a pugnacious attitude toward other cocks, was attracted by hens, and *even went so far as to tread hens as a cock.*[8]

On the whole, therefore, we may be reasonably certain that ovarian tissue engrafted into fowls, as in these experiments, may develop and preserve its functional powers to a high degree.

There is some evidence that hens with engrafted ovaries cease to lay before sister hens. During the first season they may even outstrip the normal (control) hens as far as numbers are concerned,[9] the chief constant difference being that the eggs from the operated hens are lighter in weight than normal eggs from the same varieties (Plate XXIII, Fig. 8). By the second season, the eggs are nearly normal in weight but are fewer in number—in fact but few may be laid during the second season.[10] In some cases egg production ceases at this time. One hen (W2), especially, is interesting in this connection. During the first season, 1906–7, she laid many fertile eggs. During the second season, 1907–8, she laid but a single egg. Since this time she has entirely ceased to lay. She has developed an appearance intermediate between a hen and a cock, being on the whole more feminine than masculine (Plate XXIII, Fig. 6). In

[8] It would seem to be difficult to harmonize these phenomena with the more or less definitely enunciated theory, which attributes the internal secretion of the testicles to the interstitial cells.

[9] *Jour. of Exper. Zool.*, 1908, v, 563.

[10] *Jour. of the American Med. Assn.*, 1908, li, 1314.

behavior she has developed no pronounced male characters, such as were observed in the case of Chicken 35. In view of these facts ultimate anatomical findings in her case will be of interest. Her appearance and behavior denote a loss of feminine rather than acquisition of positive masculine qualities. That is, she presents certain characters which, in the absence of *positive* feminine qualities, we regard as more or less masculine, though positive masculine qualities do not seem to be present.[11] In a way we may, provisionally at least, regard the condition as due to an unmasking of characters—judgment being normally biased by the positive feminine qualities.

(B) TESTICLES.

Results with testicles are less complete, no offspring having as yet been produced from engrafted testicles.[12] However, morphologically, the results seem as perfect as with the ovaries, engrafted testicular tissue showing a marked growth and normal structure almost four months after the operation (Plate XXIII, Fig. 7).

PROTOCOL OF CHICKEN D.—Young white cock, one-third grown, December 15, 1908. Testicles measure about 5 millimeters in the longitudinal diameter. The left testicle was removed and replaced. Two-thirds of right testicle were removed and implanted under skin at the point of left shoulder. A piece of guinea fowl testicle was implanted under the skin at the point of the right shoulder. There was an uneventful recovery.

April 5, 1909.—Fowl seems normal; it is killed. In a depression under the point of the left shoulder between the muscles and a thick layer of fat is found transplanted testicle imbedded in fat, but having a well-developed blood supply. The tissue mass is several times larger than when transplanted. On the right side is found a subcutaneous scar at the site of the implanted guinea fowl testicle. The fowl is opened. On the left side, the testicle is apparently normal in size and connections, and is light in color. A ligature used in fastening it is plainly visible. On the right side is found a small mass of testicular tissue, which is the fragment left at the time of the operation. All three masses of tissue appear to have developed to the same extent.

On histological examination all the tissues appear normal and contain numerous spermatozoa.

As yet it is undetermined whether or not testicles engrafted in the situation normally occupied by them will form connections

[11] Cf. Morgan, Experimental Zoology, New York, 1907, p. 364.

[12] Some preliminary experiments were performed with Dr. C. B. Davenport, of the Carnegie Station for Experimental Evolution, Cold Spring Harbor, in April, 1908.

with the ducts permitting of the adequate escape of semen. Therefore, proof is lacking that cocks (or rather male animals) bearing engrafted testicles can fertilize eggs in the normal manner. But since spermatazoa have been observed in abundance in engrafted testicles, even when placed under the skin, it may be possible to fertilize artificially. We have recently introduced juice, obtained from such testicular tissue by crushing, into the oviduct of hens, but as yet sufficient time has not elapsed to permit of results. If successful fertilization is thus realized, a simple way of investigating "foster-father" influence will be opened. Also by this method we may hope to obtain some further information on the function of the accessory sexual glandular structures, such information being exceedingly scant at the present time.[13]

Data concerning the effect of engrafted testicles on the preservation of male characters in castrated males are much scantier than the corresponding information for females. In fact, most of such data consist in the observations of poultrymen, that if in castration a testicle be broken up and a fragment be left in the peritoneal cavity, the fowl will probably preserve masculine characters in a higher degree than a completely castrated bird or capon. Similar observations were made by Shattuck and Seligman.[14] Lode observed excellent preservation of anatomical structure, including masses of spermatozoa in testicles engrafted subcutaneously in abnormal situations in cocks.[15] Our own results confirm this observation.

Results of iso- or hetero-engrafting of testicles are by no means so extensive nor so conclusive as in the case of ovaries. So far we have operated on too few animals to permit of any conclusive deductions, but we would predict results parallel to the results obtained on ovaries for iso- and hetero-grafts, *i. e.*, a large percentage of successful results under optimum conditions for the former, while for the latter slight, if any success.

[13] According to Walker, the ova of rabbits, guinea pigs and dogs can be successfully fertilized by injecting into the vagina a mixture of sperm from the epididymis and physiological salt solution. Quoted by Tigerstedt, translated by Murlin, Text-book of Human Physiology, New York and London, 1906, p. 693.

[14] Quoted by Marshall and Jolly, *Trans. of the Royal Soc. of Edinburgh*, 1905–7, xliv, 591.

[15] Lode, *Cent. f. Physiol.*, 1896, x, 3.

The results of all investigators, with few exceptions, point in this direction. As exceptions, McCone's claim that he successfully engrafted a dog's ovaries into a rabbit, and Schultz's claim[16] that he successfully engrafted guinea pigs' ovaries into moles will serve as examples. So far our endeavors to engraft testicles between chickens and guinea fowls have been uniformly unsuccessful, as well as all attempts to exchange other tissues (ovaries of embryo, thyroid and parathyroid, liver, spleen, kidney, adrenal, etc.) between different species, such as chicken and pigeon, pigeon and snake, white mouse and rat, or even between distantly related varieties, such as white mouse and common wild mouse, white mouse and field mouse, etc. These experiments have as yet yielded no positive evidence that such transplantations can be successfully performed. But it would be a mistake to discontinue investigation in this direction on the strength of such results since a better knowledge of conditions may permit of the execution of such experiments under conditions more nearly approaching the optimum. If this improvement of conditions be realized, and it is by no means improbable, we may hope for an improvement in the results.

In certain of the subcutaneous grafts, superficial fat was so abundant that it may have acted as a factor hindering survival of the tissues. In all markedly successful transplantations, the tissues have invariably been found in close relations with good sized blood vessels, while in all cases where such tissues have been found to have survived but to have made but little or no growth, they have been more remotely situated from such blood vessels. For example, tissues successfully placed anterior and internal to the point of the shoulder in fowls have shown much better growth than similar tissues placed beneath the skin on the point of the shoulder. Blood vessels are numerous in the former situation, while in the latter, none are readily visible. It would seem, therefore, that such considerations (presence of fat, vascularity) are of importance in determining the site for a simple graft. Other factors, such as age, sex, season, toxicity, etc., will be treated in a later paper.

Transplantations of ovaries and testicles with anastomosis of the

[16] Marshall and Jolly, *loc. cit.*, pp. 593–4.

FIG. 1.

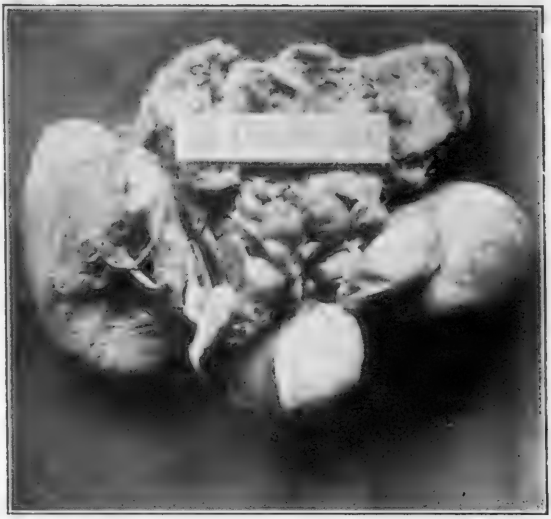
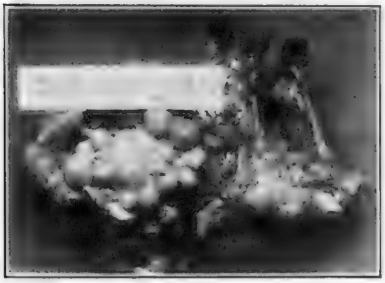


FIG. 2.

FIG. 3.



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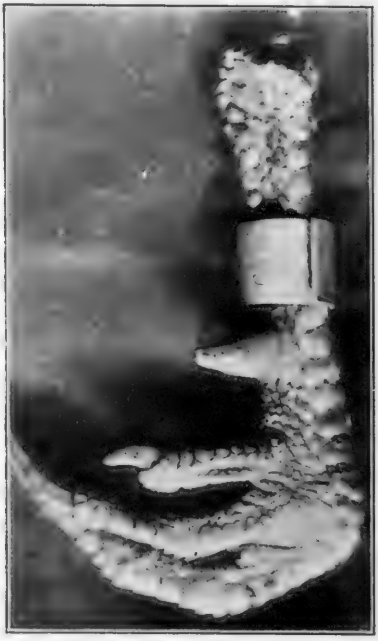
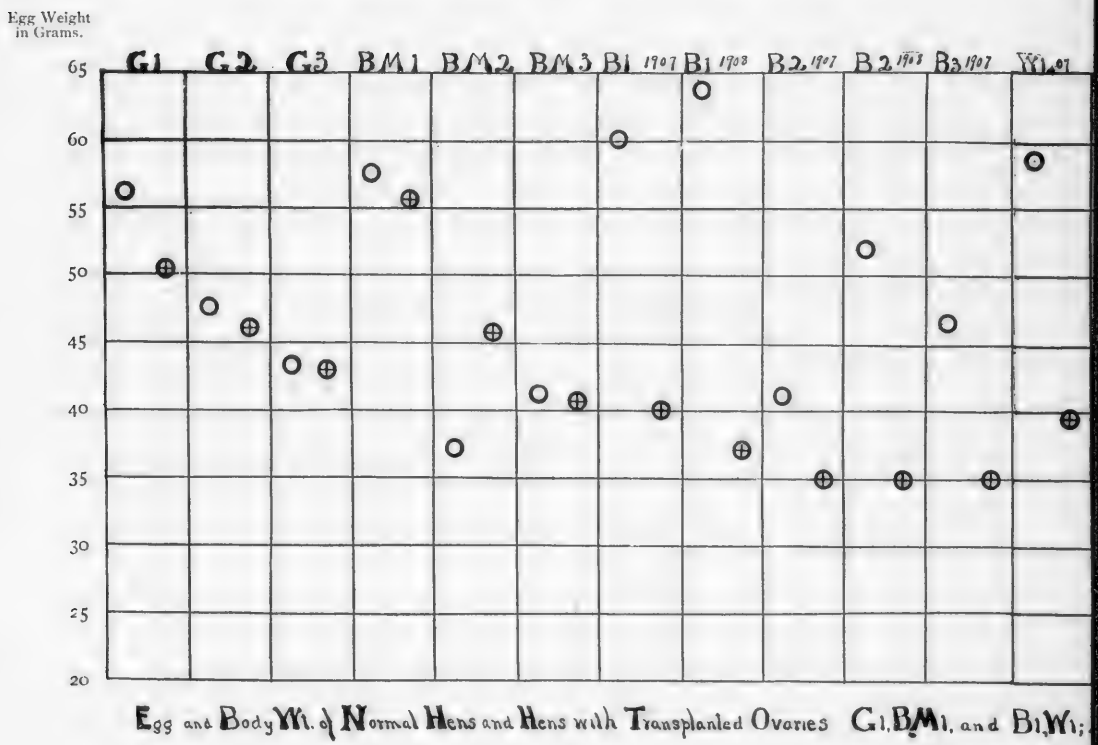


FIG. 5.

FIG. 6.



Egg and Body Wt. of Normal Hens and Hens with Transplanted Ovaries G1, BM1, and B1, W1;

FIG. 8.

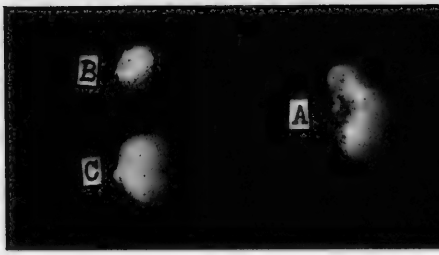

B
A
C

FIG. 7.

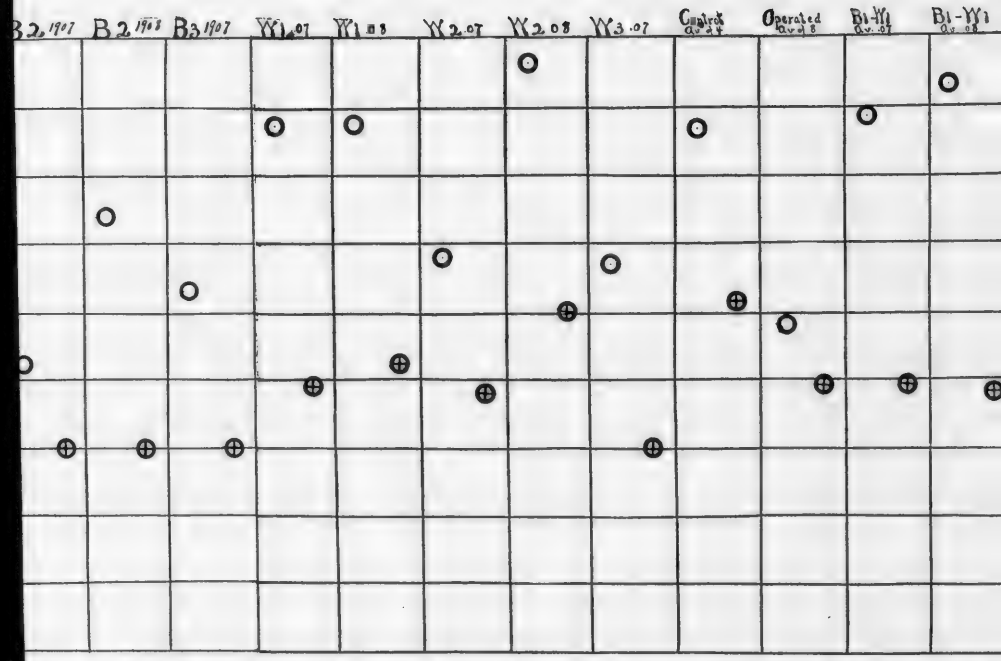
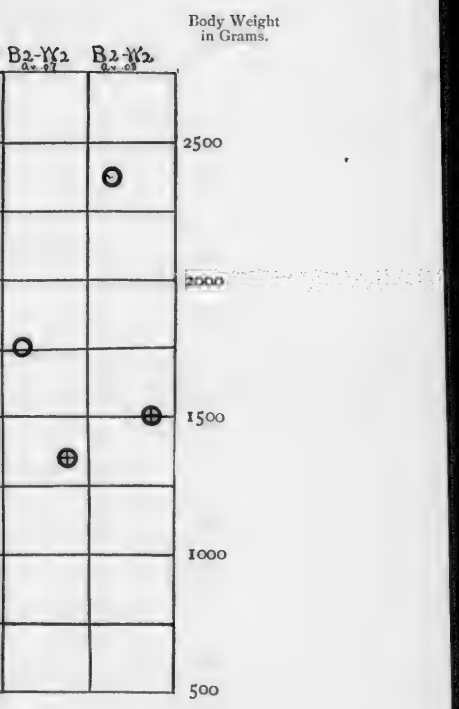

B2 1907
B2 1908
B3 1907
W1 07
W1 08
W2 07
W2 08
W3 07
Control
Operated
B1-W1
B1-W1
aries G1,BM1, and B1,W1; Normal; all others Operated.
=Egg wl;
=B

FIG. 8.

PLATE XXIII.



nutrient blood vessels have not been practiced sufficiently to permit of comparisons or conclusions. As the technique of such operations involves a description of blood vessel anastomosis, the reader is referred to former publications on the subject.[17]

Since results are readily obtained in certain animals hitherto employed by the simple method of transplanting, there has been no need for the more complicated operation. But there are good reasons for believing that in larger and older animals, as well as under less favorable conditions, successful results will perhaps be more certain after transplantation with anastomosis of the blood vessels than after the simpler operation.

EXPLANATION OF PLATES.

PLATE XXII.

FIG. 1. Light Brahma No. 2. December 15, 1908, ovary transplanted subcutaneously. Removed one-third and photographed, April 7, 1909.

FIG. 2. Ovary from normal laying hen.

FIG. 3. Engrafted ovary from laying hen.

FIG. 4. Engrafted ovary from pullet, before laying.

FIG. 5. Foot of fowl No. 35.

PLATE XXIII.

FIG. 6. W 2, White Leghorn carries ovary from Black Leghorn, B 2.

FIG. 7. Chicken D. A, Left testicle removed and reimplanted at original site. B, Fragment of right testicle left *in situ*. C, Portion of right testicle removed and implanted under skin of left shoulder. Operation, December 15, 1908; specimens taken, April 5, 1909.

FIG. 8. Chart showing egg and body weights.

[17] *Science*, N. S., 1909, xxix, 29; *Interstate Medical Journal*, 1908, xv, 489; *Jour. of the American Medical Assn.*, 1908, li, 1658–62.

THE CHANGES IN THE VENOUS BLOODFLOW ON ADMINISTRATION OF AMYL NITRITE.[1]

By R. BURTON-OPITZ and H. F. WOLF.

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PLATE XXIV.

The question as to whether the characteristic changes in general blood-pressure resulting from the introduction of amyl nitrite are caused by a direct inhibitory influence upon the plain muscle cells of the peripheral vascular channels or whether they are referable to an action of this drug upon central nervous structures has been investigated repeatedly. By stimulation of the splanchnic nerve, Bernheim[2] succeeded in raising the blood-pressure of animals poisoned with amyl nitrite, and concluded, therefore, that the contractile elements of the blood vessels are not affected by the drug. The view that amyl nitrite exerts preëminently a central influence is also shared by Filehne.[3] Atkinson[4] having established an artificial circulation through the aorta, observed a more ready outflow of fluid whenever amyl nitrite was added to the perfusion liquid. Under similar conditions, Marshall[5] obtained an increase of flow through the excised kidney.

A ready means of studying the influence of amyl nitrite upon the circulation seemed to be furnished by the recording stromuhr. With the help of this instrument, we thought it possible to follow accurately the diverse quantitive changes of the blood-stream which this drug might be capable of producing, and to bring these changes at the same time into relation with the variations in blood-pressure.

We selected the capillary field of the femoral artery for the de-

[1] Received for publication January 25, 1910.

[2] *Arch. f. d. ges. Physiol.*, 1874, viii, 253.

[3] *Arch. f. d. ges. Physiol.*, 1874, ix, 470.

[4] *Jour. of Anat. and Physiol.*, 1888, xxii, 236.

[5] *Jour. of Physiol.*, 1898, xxii, 1.

terminations, the flow from this area being measured directly by a stromuhr[6] inserted in the femoral vein. The experiments were carried out on dogs in ether narcosis. The right femoral vein was connected with the stromuhr, while the corresponding artery and also the vein of the opposite side were isolated sufficiently to permit at any time the insertion of the needle of a small syringe. In this manner it was made possible to inject amyl nitrite either into the artery or into the vein, and hence to obtain its peripheral effect together with its central effect in the course of one determination. Inhalations of amyl nitrite were also resorted to, the inhalations being given while the normal volume of the bloodstream was being recorded. In two experiments the drug was administered by the same method while the intact leg was perfused with defribrinated blood. The blood pressure was determined in the left femoral artery and also in the right femoral vein centrally to the stromuhr. A mercury and a membrane manometer were employed for this purpose. The time was indicated by a Jaquet chronograph.

As the results of the experiments were uniform in character, a detailed account of them is not deemed necessary. To illustrate, we insert in this place the data of Experiment 3a, in the course of which an injection of amyl nitrite was made, first into the artery of the same leg and subsequently into the vein of the opposite side.

The changes outlined in this table are clearly represented in the accompanying reproduction of the record of the stromuhr (Plate XXIV, Figs. 1 and 2) written during phases 2 to 11. The hemodynamical values given above can, therefore, be transferred directly to the curve. Besides the record of the stromuhr (St), the curve also shows the blood pressure, arterial as well as venous (*AP* and *VP*) above their common abscissa. The time is marked in seconds.

Between points *A* and *B*, the bloodflow showed a value of 0.84 to 0.81 cubic centimeter per second, while the manometer recorded an arterial pressure of 88.9 millimeters of mercury. At *B*, two drops of a weak solution of amyl nitrite were injected into the femoral artery of the same leg. At *C*, after a latent period of about

[6] The recording stromuhr described by Burton-Opitz was used. See *Arch. f. d. ges. Physiol.*, 1908, cxxi, 150.

EXPERIMENT 3A.

Effect of Amyl Nitrite upon Venous Blood-flow. Weight of Dog, 12.5 Kilograms.

Phase of stromuhr.	Duration in seconds.	Total quantity in c.c.	Quantity in c.c. per second.	Blood-pressure in mm. Hg.		Remarks.
				Vein.	Artery.	
1	23.6	20.0	0.84		89.8	
2	24.4	20.5	0.84		—	
3*a*	15.4	11.6	0.81	8.2	88.9	
3*b*	9.2	9.0	0.97	8.4	88.8	Injection into artery of same side.
4	9.4	20.0	2.12	12.6	—	
5	6.3	19.5	3.09	19.5	—	
6	7.2	19.5	2.70	19.2	86.0	
7	9.1	20.0	2.19	13.5	87.5	
8	10.7	19.4	1.81	13.0	—	
6	13.1	19.4	1.48	10.0	—	
10	16.1	19.6	1.21	9.5	88.5	
11	20.0	19.6	0.98	9.0	—	
12	24.1	20.0	0.82	—	58.8	Injection into vein of opposite side.
13	28.9	19.6	0.67	—	56.4	
14	28.0	19.5	0.69	8.8	56.0	
15	25.8	19.6	0.75	9.0	64.5	
16	25.1	19.6	0.78	—	72.6	
17	17.5	14.6	0.83	—	88.0	

five seconds, the venous bloodflow steadily assumed a greater volume until it attained its maximal value of 3.09 cubic centimeters per second during phase 5, at about *D*. During phases 6 to 11, a gradual return to normal can readily be observed.

The quantitive changes here indicated betray first of all the vasodilation occurring within the area of the femoral arterioles and the subsequent rush of blood from the arterial into the venous channels. This local venous engorgement is accompanied by an increase in venous pressure, the rise beginning at *C* and ending at about *D*, at which point a maximal pressure of 19.5 millimeters of mercury was registered. The general arterial blood-pressure, measured in the femoral artery of the opposite leg, remained practically unaltered, in spite of the fact that such pronounced changes as those just described were being enacted in a neighboring vascular area. Naturally, the magnitude of this vaso-motor reaction preserves a definite relationship to the amount of amyl nitrite injected. In the experiment here cited, just enough of the drug was used to produce a

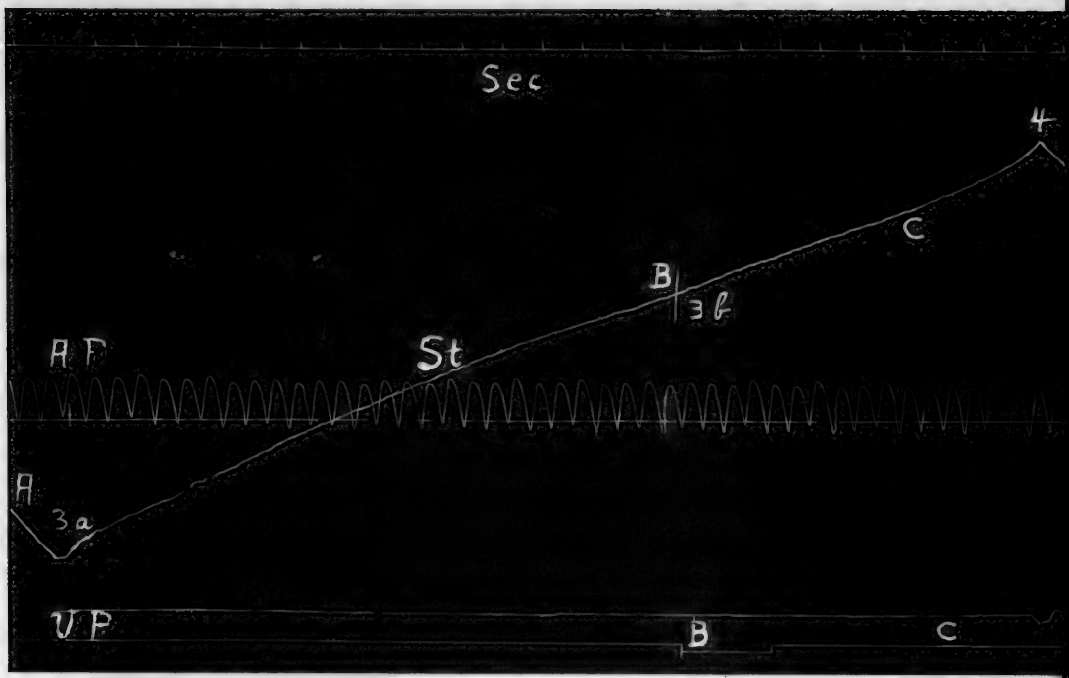


FIG. 1.

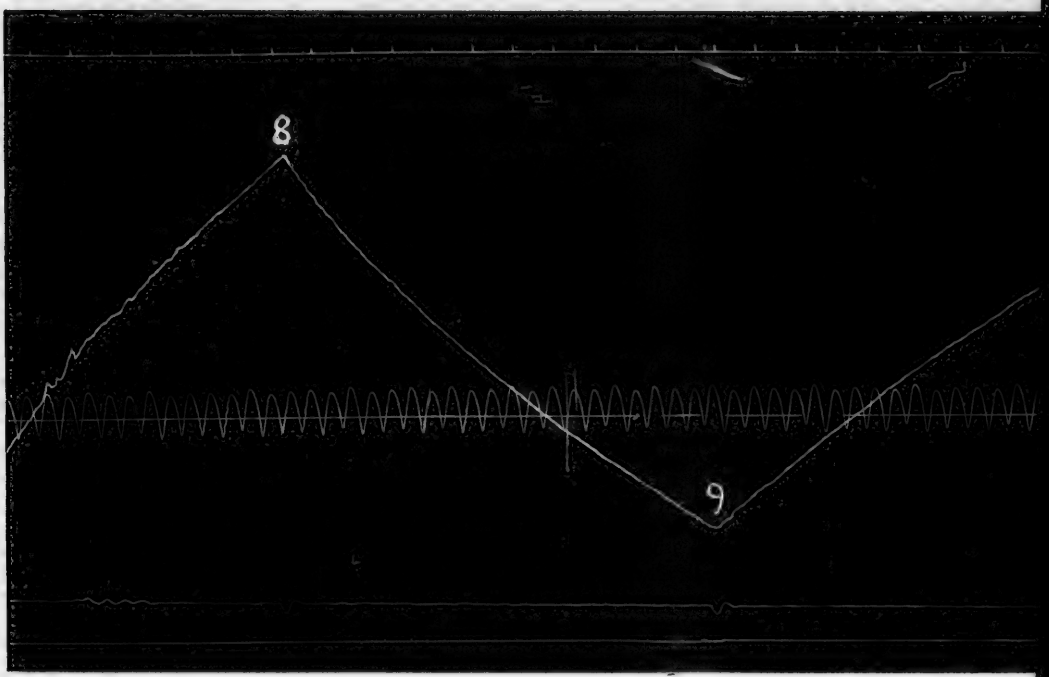


FIG. 2.

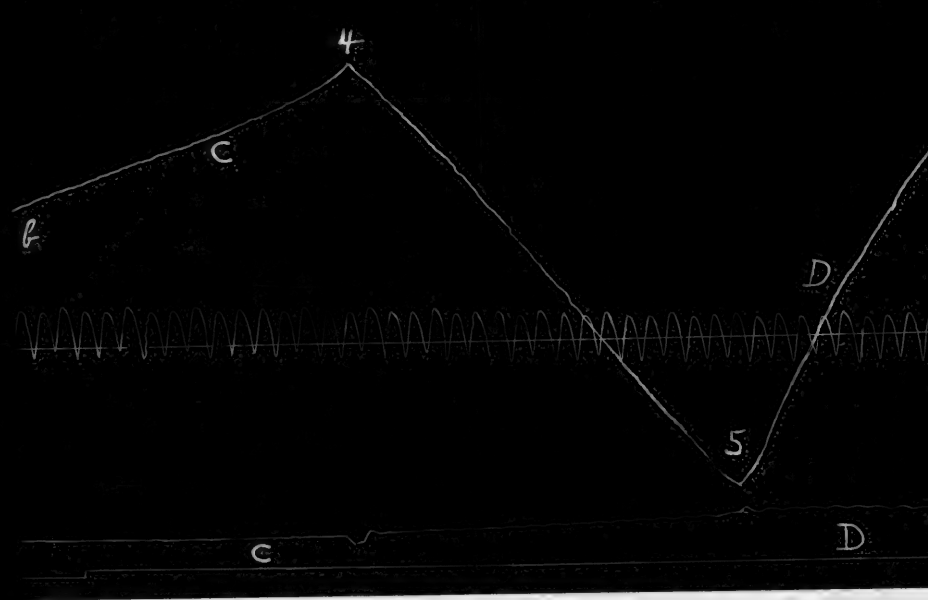


FIG. 1.

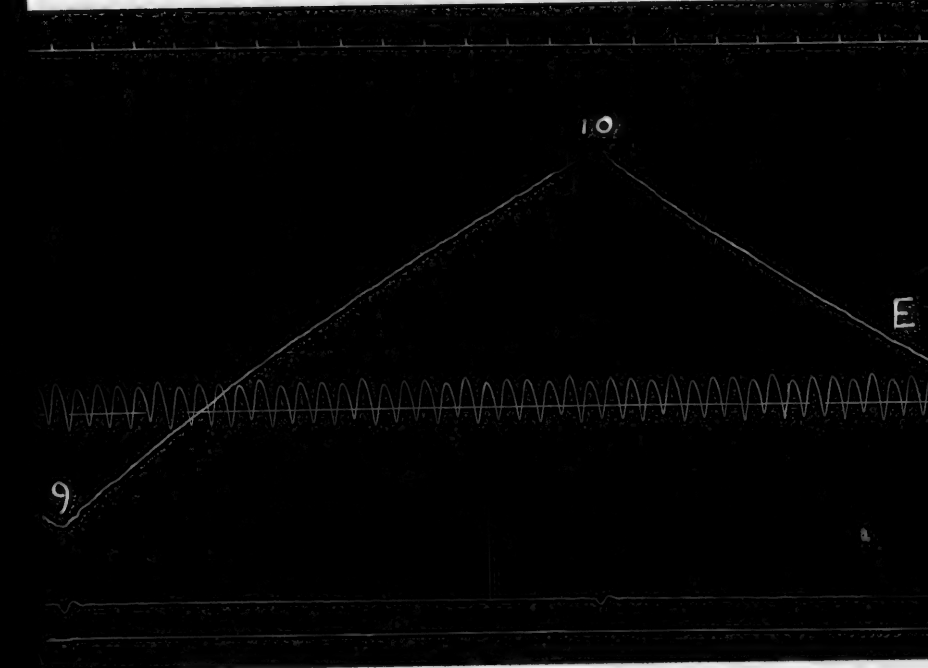
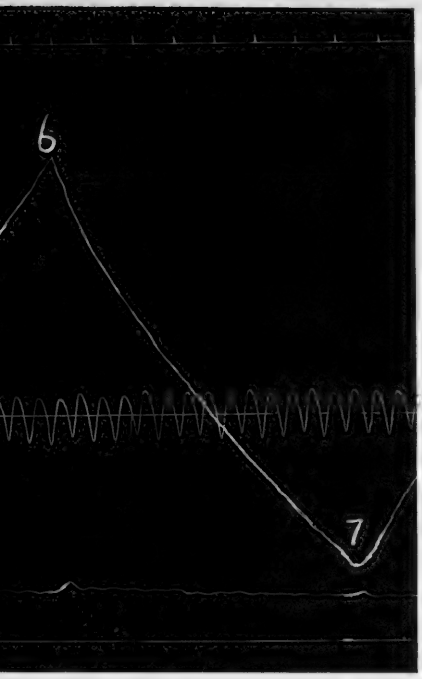


FIG. 2.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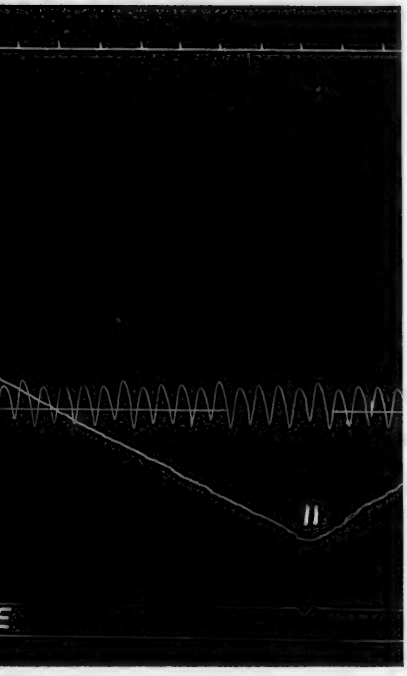
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moderate reaction, so that its power was very nearly spent in the production of the peripheral dilatation. That only a minimal amount of it remained effective and escaped into central circulatory channels, is indicated by the very slight fall in general arterial pressure occurring at *D, i. e.,* at a time when the vascular conditions in the right leg again tended to assume a normal character.

It must be evident that if an effective amount of the drug is carried from its place of injection in the artery through the capillaries, first into central and then into peripheral channels, changes of quite a different type must follow the local vaso-dilation. Under these conditions, the character of the vascular reaction would be identical with the reaction resulting from the introduction of the drug into the vein of the opposite leg.

Whenever the latter procedure was resorted to, or whenever the amyl nitrite was administered by inhalation, the venous bloodflow showed a slight decrease, occurring simultaneously with the fall in arterial blood-pressure. The lessened venous bloodflow, therefore, must be dependent in this case upon the reduction in arterial tonicity.

While the amyl nitrite invariably produced a marked lessening of the arterial blood-pressure, the venous pressure retained its normal value in all cases in which the drug was permitted to exert a general effect of ordinary magnitude. When an artificial circulation through the normal leg was established and inhalations of amyl nitrite were given, no changes in the flow of the perfusion fluid could be detected. It appears, therefore, that the vaso-dilatory effects of this drug are not incited with the help of central nervous structures.

CHANGES INDUCED IN THE BLOOD OF RABBITS BY LIVING IN AN ATMOSPHERE OF WATER GAS.[1]

By GEORGE G. NASMITH, Ph.D., and F. C. HARRISON, B.A., M.B.

(From the Ontario Health Laboratories, Toronto.)

Three years ago one of us,[2] in the attempt to produce anemia in guinea pigs by carbon monoxide, found that certain blood changes of rather a surprising nature were produced. Animals were placed in a chamber through which was drawn a mixture of water gas, containing about 20 per cent. of carbon monoxide, and air, and they were kept continuously in that atmosphere. After a few days it was found that the number of erythrocytes in the peripheral circulation began to increase until a definite maximum had been attained, the maximum varying according to the proportion of the blood hemoglobin saturated with carbon monoxide. Thus, when 25 per cent. of the hemoglobin was saturated, the number of erythrocytes present in the peripheral or internal circulation was found to be 8,000,000 as compared with the normal number of 6,000,000. Other changes were also noted which were constant and definite in character.

We considered it of interest to find out whether, in the blood of animals subjected to the continuous action of water gas, hemolysins, agglutinins or precipitins would be formed which would react with the sera and erythrocytes of normal rabbits. We also wished to see whether we could produce anemia by means of carbon monoxide by varying the amount and method of giving the same. We selected rabbits for these experiments, as we needed to draw small amounts of blood at frequent intervals. It was, therefore, necessary to see whether rabbits would react to the carbon monoxide in the same way as guinea pigs.

Rabbits were accordingly placed in the gas chamber through which a dilute mixture of gas and air was drawn for the first

[1] Received for publication January 17, 1910.

[2] Nasmith and Graham, *Jour. of Physiol.*, 1906, xxxv, 32.

twenty-four hours; a larger percentage of gas was then added, until on the third day about 25 to 33 per cent. of their hemoglobin was saturated. If placed directly into this strength of gas they invariably died. At the end of three days profound degenerative changes were found in the erythrocytes, corresponding in detail to those found in the guinea pigs. Nucleated reds were always found, their appearance usually occurring within two days. The changes in the white blood corpuscles were also comparable to those in the guinea pig.

A rabbit (B and W) was placed in the gas chamber on January 4, 1909.

RABBIT B AND W.

Date.	R.B.C.	W.B.C.	Per cent. hemoglobin.	Nucleated red corpuscles.
Jan. 4, '09	7,008,000	10,400	79	0
" 8, "	6,832,000	33,500	79	141 in a 500 differential count
" 29, "	10,704,000	4,600		
Feb. 2, "	10,976,000	7,300	118	1

Other animals have reacted in exactly the same way, so the red blood corpuscles of the rabbit evidently are affected by carbon monoxide in the same way as those of the guinea-pig.

Rabbits with a high erythrocyte content have also had the blood from the carotid and heart counted and the same number has been found there as in the peripheral blood. The increase, therefore, as in the guinea pig is a real increase in the production of red blood corpuscles and is not due to a mere peripheral stasis. The hemoglobin increase, though marked, does not correspond to the rise in erythrocytes. Thus a rabbit with 11,000,000 erythrocytes only had 118 per cent. hemoglobin; the increase in the erythrocytes being 82 per cent., while the increase in hemoglobin was only 37 per cent. The specific gravity of the blood of a rabbit with a high erythrocyte count is greatly increased. For instance the blood of Rabbit L with 11,272,000 erythrocytes had a specific gravity of 1,072, while a normal rabbit with 6,000,000 erythrocytes had a specific gravity of only 1,051. It was found also that the erythrocytes of a rabbit with a high blood count had the same osmotic tension as erythrocytes of normal rabbits. This was shown by adding the washed corpuscles

to varying strengths of sodium chloride solution. The corpuscles from rabbits with high and normal erythrocyte counts were hemolyzed by exactly the same strength of sodium chloride solutions, so there was evidently no difference in their osmotic tension.

Taken altogether it has been found by us that rabbits living continuously in an atmosphere of water gas react in exactly the same way as do guinea pigs, and the changes as far as the red blood corpuscles are concerned, are quite similar. Furthermore, the conclusion that we drew previously, namely, that the increase of erythrocytes is due to an attempt on the part of the animal to keep its oxygen-carrying capacity constant, we believe still holds good. We endeavored to determine whether the law of Weigert would hold under these circumstances, that is, whether animals undergoing active regeneration of red blood corpuscles would go on forming new corpuscles after they had been removed from the gas—in other words, when the stimulus had been withdrawn.

A rabbit with 11,000,000 erythrocytes per cubic millimeter was bled from the jugular vein, forty cubic centimeters of blood being withdrawn. The counts from the marginal ear vein taken at various times since the operation are given below.

Date.	Erythrocytes.	Per cent. of hemoglobin.
Mar. 6, 09	11,512,000	120
Mar. 8, 09	8,360,000	95 two days after operation
Mar. 10, 09	9,264,000	—
Mar. 15, 09	9,336,000	94
Apr. 1, 09	9,330,000	—
Apr. 12, 09	9,784,000	—

A second rabbit similarly treated gave the following results:

RABBIT B AND W.

Date.	Erythrocytes.	Per cent. of hemoglobin.
Feb. 26, 09	10,976,000	118
Mar. 2, 09	9,592,000	— day after operation
Mar. 3, 09	8,040,000	92
Mar. 8, 09	9,784,000	92
Apr. 17, 09	9,596,000	87
Apr. 26, 09	7,184,000	—

From these experiments it may be seen that two days after the operation, in both animals there appeared to be a diminution in the

number of erythrocytes, then a rise of about a million, the total count then remaining constant for several weeks, although the hemoglobin had fallen back to its normal amount. Other rabbits taken from the gas when undergoing active regeneration of erythrocytes and placed in air show no further increase in the numbers of erythrocytes. Evidently, therefore, in these cases the blood-forming organs cease to throw out large numbers of erythrocytes into the circulation just as soon as the stimulus has been withdrawn. In other words, there is only a sufficient amount of hemoglobin produced and distributed among the erythrocytes to carry the amount of oxygen required by the tissues. The fact that with a definite saturation of the blood with carbon monoxide there is a definite quantity of corpuscles and hemoglobin produced for that saturation is another proof that the stimulus acts only until certain conditions for the supplying of oxygen to the tissues are fulfilled. The hydrogen, methane and carbon dioxide present in the gas mixture would all tend to have the effect of reducing the oxygen tension in the lungs. Rabbits given the gas air mixture for three hours followed by air for three hours for periods of days or weeks reacted in exactly the same way as did rabbits living continuously in the gas air mixture.

An animal, Rabbit M, was placed in the gas mixture and kept there for four days until the blood showed a great amount of erythrocyte degeneration, with many nucleated red blood corpuscles. The animal was then operated on and blood smears taken from the carotid, splenic vein, portal vein and hepatic vein. Differential counts of these smears did not show any marked change, that is, there was no apparent difference between the blood going to and coming from the liver and spleen. Nor was there any perceptible difference between the numbers of degenerated erythrocytes going to and coming from the spleen and liver.

As it was thought possible that there might be something of the nature of hemopoeitin present in the blood of rabbits which were rapidly producing new erythrocytes, some of them were bled to death and the serum injected into fresh rabbits which had had their erythrocytes accurately counted. In no case have we been able to demonstrate satisfactorily anything of the nature of hemopoeitin,

DIFFERENTIAL COUNTS OF RABBIT M.

Source of blood.	Pseudoeosinophils.	Small lymphocytes.	Large lymphocytes.	Eosinophils.	Basophils.	Nucleated erythrocytes met with in a 500 count.	Transitionals.
Ear	38.4	49.8	1.4	4.0	6.4	50	
Carotid	33.8	57.8	1.1	3.0	4.3	14	
Splenic vein	40.8	49.0	2.0	4.2	4.0	41	
Portal vein	47.0	42.2	5.6	2.0	3.0	23	.2
Hepatic vein	39.4	45.2	8.2	4.0	3.0	32	.2

although occasionally there has been a slight rise in the erythrocyte count; in other cases there has been a slight fall.

For example, Rabbit YF was injected subcutaneously with 25 cubic centimeters of serum obtained from a younger rabbit which had been in the gas for four days and showed marked erythrocyte degeneration with many nucleated reds present.

Date.	Erythrocytes.
June 22, 09	7,024,000—injected with 25 c.c. serum
June 23, 09	7,032,000
July 3, 09	7,296,000

These experiments also serve to demonstrate the absence of hemolysins, for, though there was sometimes a slight reduction in the number of erythrocytes, there was no constancy in the results obobtained.

Washed corpuscles from normal rabbits were incubated with serum obtained from the blood of rabbits showing marked degeneration of the erythrocytes. Hemolysis was never obtained in the several cases investigated, and we have, therefore, concluded that in the blood of rabbits showing severe erythrocyte degeneration hemolysins capable of hemolysing rabbit erythrocytes are not present. We have also tested the washed corpuscles of normal rabbits with serum from rabbits in various stages of erythrocyte degeneration and regeneration but we have never been able to demonstrate the presence of agglutinins. Similarly, we have tested normal rabbit sera against sera of rabbits in various stages of degeneration and regeneration and have never been able to show the presence of precipitins.

The fact that we were unable to produce hemolysis of the washed rabbit corpuscles with salt solutions of other than the strength re-

quired to hemolyze normal washed corpuscles, although the specific gravity of the blood in rabbits with a ten million erythrocyte count was much above the normal, 1.070 compared with 1.049, seems to demonstrate the fact that the plasma of both rabbits must be of the same specific gravity. In other words the increase in the specific gravity of the blood is due to the additional corpuscles and hemoglobin only.

Conclusions.

1. Rabbits living in an atmosphere of carbon monoxide react in general as guinea-pigs react.

2. The increased specific gravity of the blood in "carbon monoxide" rabbits is due wholly to the increase in the number of erythrocytes and of hemoglobin, and not to any change in the plasma.

3. Weigert's law of inertia, that production would continue after the stimulus has ceased to act, does not hold in the case of carbon monoxide stimulated rabbits.

4. Hemolysins, precipitins and agglutinins towards normal rabbit corpuscles and sera were not present in carbon monoxide rabbits.

5. "Hemopoeitin" or anything of a similar nature was not found in rabbits in any stage of erythrocyte degeneration or regeneration.

STUDIES IN EDEMA.

VI. The Influence of Adrenalin on Absorption from the Peritoneal Cavity, with Some Remarks on the Influence of Calcium Chloride on Absorption.[1]

By MOYER S. FLEISHER and LEO LOEB.

(From the Laboratory of Experimental Pathology, University of Pennsylvania.)

In some earlier papers (1, 2, 3) on the production of peritoneal transudates in rabbits by the intravenous infusion of large quantities of sodium chloride solution or of mixtures of a sodium chloride and calcium chloride solution, or of these solutions with the addition of adrenalin, we found that both calcium chloride and adrenalin increased the amount of peritoneal transudate. We pointed out that the increased amount of peritoneal transudate could be due to two conditions, namely, either an actual increase in the transudation under the influence of these substances, or a decrease in the rapidity of absorption of fluid from the peritoneal cavity, or to a combination of these factors. It was, therefore, necessary for us to determine the influence of adrenalin and calcium chloride on absorption.

We used the following method of experimentation: we injected definite quantities of 0.85 per cent. sodium chloride solution, warmed to body temperature, into the peritoneal cavity of rabbits, by means of a syringe and needle, especial care being taken not to injure the intestines. In the individual experiments concerning the influence of calcium chloride, the amounts of fluid injected into the peritoneal cavity varied between 110 and 150 cubic centimeters. However, in all experiments relating to the influence of adrenalin on absorption, 120 cubic centimeters of 0.85 per cent. sodium chloride solution were injected. After the injection of the fluid, and during the period of the experiment, the rabbit was in no way restrained, being

[1] Received for publication January 12, 1910.

allowed to move about freely in its cage. After a period of time, which in the experiments with calcium chloride varied between two and three hours, but which in the other experiments was always two hours and a half, the animal was killed, and the fluid was recovered from the peritoneal cavity by widely opening the abdomen and draining the fluid contents into a dish or beaker.

THE ABSORPTION OF SODIUM CHLORIDE AND SODIUM CHLORIDE-CALCIUM CHLORIDE SOLUTIONS FROM THE PERITONEAL CAVITY.

In these experiments with sodium chloride and sodium chloride-calcium chloride solutions, our aim was merely to determine the comparative absorption of fluid as influenced by these solutions. Thus, we carried out experiments in which two animals of equal weight were injected with equal quantities of either sodium chloride or sodium chloride-calcium chloride solutions; in every pair of animals of equal weight injected with equal quantities of fluid, the duration of the experiment was the same.

In sixteen experiments in which one animal was injected with 0.85 per cent. sodium chloride solution and another animal with a mixture of four parts of 0.85 per cent. sodium chloride and one part of 1.22 per cent. calcium chloride solution, we found the average amount of fluid recovered from the peritoneal cavity in the first series to be 59 cubic centimeters; in the second, 64 cubic centimeters. We can, therefore, conclude that the addition of calcium chloride, in such an amount as we have used in our experiments, to sodium chloride solution has an insignificant action on absorption. It possibly delays absorption to a very slight degree. The difference here is within the limits of experimental error. It is, therefore, not possible to account for the considerable differences in the quantities of peritoneal transudate noted after the intravenous infusion of sodium chloride solution and sodium chloride-calcium chloride solution through the influence of calcium chloride upon absorption from the peritoneal cavity; but we must consider this increase of peritoneal transudate under the influence of calcium chloride as due to a direct influence on the transudation into the peritoneal cavity.

We found many individual variations in the amounts of fluid

absorbed; it was therefore necessary to make a large series of experiments and to take the mean. However, notwithstanding individual variations, if, in considering a large series of experiments, one compares the mean of the whole series with the mean of six or seven successive experiments of the series, it will be found that the two are very similar.

For this reason we feel quite confident that notwithstanding the individual variations, our experiments permit us to draw definite conclusions and this applies also to the following series of experiments.

THE INFLUENCE OF ADRENALIN UPON ABSORPTION FROM THE PERITONEAL CAVITY.

In order to determine the influence of adrenalin upon absorption from the peritoneal cavity, we carried out two series of experiments, which differed in the manner in which adrenalin was administered. In one series, we injected 0.25 cubic centimeter of adrenalin (Parke, Davis and Co., 1 : 1000 solution) into the peritoneal cavity, every thirty minutes after the sodium chloride solution had been injected. Thus, a total of 1.25 cubic centimeters of adrenalin was administered. In another series, the adrenalin was injected intravenously. Usually, 0.2 cubic centimeter was given at each injection. In the majority of cases, four injections were given, at intervals of thirty minutes; when the rabbits appeared to be very much affected by the injection, a longer interval was allowed between the injections. In the majority of cases, a total of 0.8 cubic centimeter of adrenalin was injected in these animals. In all these experiments, a constant amount of fluid (120 cubic centimeters) was injected into the peritoneal cavity; and the experiment extended over a period of two hours and a half. The animals used in these experiments had not received any food for twenty-four hours previous to the experiment.

In a third series of experiments, instead of 120 cubic centimeters of 0.85 per cent. sodium chloride solution, 120 cubic centimeters of distilled water were injected into the peritoneal cavity of rabbits. Half of these were used as controls, while the other half received intraperitoneal injections of adrenalin, in the same quantity as in

the experiments in which sodium chloride was used. In a fourth series of experiments, 120 cubic centimeters of a 1.5 per cent. sodium chloride solution were injected intraperitoneally; in this series also control and adrenalin experiments were made.

In a series of thirty-one control experiments,[2] in which 0.85 per cent. sodium chloride solution was injected into the peritoneal cavity, we recovered, after a period of two hours and a half, an average of 77 cubic centimeters of fluid, and collected an average of 11 cubic centimeters of urine. In twenty-nine experiments in which adrenalin was injected intraperitoneally in addition to the 0.85 per cent. sodium chloride solution, an average of only 41 cubic centimeters of fluid was recovered; and an average of 41 cubic centimeters of urine was excreted by the animals during the period of the experiment.

In a series of 20 experiments, adrenalin was injected intravenously and in these experiments, we recovered an average of 62 cubic centimeters of fluid, and collected an average of 11 cubic centimeters of urine. In almost every experiment of this last series, the animal was very markedly affected by the adrenalin, showing weakness and dyspnea. It may be noted that adrenalin caused no increased diuresis in this series, while, when injected intraperitoneally, its diuretic action is marked. In experiments in which, instead of 0.85 per cent. sodium chloride solution, we injected the same amount of distilled water (120 cubic centimeters) into the peritoneal cavity, we recovered in a series of thirteen control animals, an average of 44 cubic centimeters of fluid while in the same number of animals in which, in addition to the distilled water, adrenalin was injected intraperitoneally, only 21 cubic-centimeters of fluid were recovered. In the latter cases, an average of 49 cubic centimeters of urine was excreted; in the former an average of fourteen cubic centimeters of urine was collected.

When 120 cubic centimeters of a 1.5 per cent. sodium chloride

[2] In view of the fact that the experimental conditions in the series of experiments comparing the absorption of sodium chloride and sodium chloride-calcium chloride solutions differ from the conditions in these experiments both as regards the duration of the experiments and the amounts of fluid injected, there exist some differences in the averages of the amounts of recovered fluid in the two cases.

solution were injected intraperitoneally, we recovered, in a series of six experiments, an average of 116 cubic centimeters of fluid. Thus, almost no fluid had been absorbed. These animals secreted, during the period of the experiment, an average of 8 cubic centimeters of urine. When adrenalin was injected intraperitoneally, in addition to the hypertonic chloride solution, we recovered an average of 81 cubic centimeters of fluid and found an average of 30 cubic centimeters of urine secreted. Thus, in every case the injection of adrenalin has increased to a very marked extent the absorption of fluid from the peritoneal cavity.

Exner (4) and Meltzer and Auer (5) have held that adrenalin delays the absorption from the peritoneal cavity. Exner found that the intraperitoneal injection of adrenalin delayed the absorption from the peritoneal cavity of strychnine, potassium cyanide, physostigmin, and various emulsions, such as liquid paraffin or proteus vulgaris cultures. He believed that adrenalin delayed the absorption through the lymphatics, but did not affect the absorption through the capillaries. Meltzer and Auer have shown that strychnine and fluorescine are more slowly absorbed from the peritoneal cavity when adrenalin is injected subcutaneously or intravenously and they believe that adrenalin increases the tonicity of the endothelial cells of the peritoneum and of the bloodvessels, thus causing a narrowing the "stomata" between the cells.

Achard and Caillard (6) found that when, in addition to a solution of glucose, adrenalin was injected into the peritoneal cavity of a guinea-pig, very slightly less fluid was found here at the conclusion of the experiment than in control experiments. They also found that less sodium chloride had passed into the peritoneal fluid when adrenalin was injected intraperitoneally, and that less glucose was absorbed. When adrenalin was injected subcutaneously the processes of absorption were left unchanged. These observers, however, performed only one experiment of each kind.

The experiments both of Exner and of Meltzer and Auer[3] dealt

[3] Since the appearance of our preliminary report (*Proc. of the Soc. for Exper. Biol. and Med.*, 1909, vi, 115), Falta and Jvcovic (*Berliner klin. Woch.*, 1909, xlvi, 1929), have stated that the influence of adrenalin in preventing the appearance of the symptoms of strychnine poisoning is not due to a delayed absorption of this substance but to a "biological antagonism" between adrenalin and strychnine.

mainly with the absorption of dissolved substances, and not with the absorption of the solvent (water); and since we had obtained results contrary to those obtained by these observers when we studied the influence of adrenalin on the absorption of fluid, we determined in a number of experiments the amounts of sodium chloride in the fluid recovered from the peritoneal cavity. We were thus able to determine the amount of sodium chloride absorbed from the peritoneal cavity. In addition, we determined the sodium-chloride content of the blood serum[4] and also of the urine. For the determination of the chlorides, Volhard's method was used.

In some of these experiments, the osmotic pressure of the blood-serum and of the recovered peritoneal fluid was also determined with Beckmann's freezing point apparatus.

According to most observers, the freezing point of the blood serum of normal rabbits varies between — 0.56° and — 0.60°. In three normal animals, we found it to be — 0.60°. Two hours and a half after the injection into the peritoneal cavity of 0.85 per cent. sodium chloride solution, part of which had been absorbed, we found the average lowering of the freezing point in the serum of both control animals and of animals intraperitoneally injected with adrenalin to be — 0.583°. In animals which received adrenalin intravenously, the average freezing point was — 0.593°. The freezing point of the recovered peritoneal fluid averaged the same in all three series of experiments, being — 0.593°. Thus, in experiments in which adrenalin was injected intravenously, the osmotic pressure of the serum after absorption of sodium chloride solution was slightly greater than in control animals or in animals in which adrenalin was injected intraperitoneally. The osmotic pressure of the peritoneal fluid is usually higher than that of the blood; this applies to both the individual cases and to the averages. After the intravenous injection of adrenalin, it is identical in both fluids.

[4] We obtained the serum by bleeding the rabbit. The blood was whipped, in order to defibrinate it; and the serum was separated from the corpuscles by centrifugalizing. It appears that the serum obtained from blood that is exposed to the air during defibrination has a slightly different sodium chloride content from that of the serum obtained from clotted blood or blood that is not exposed to the air during defibrination.

ABSORPTION FROM THE PERITONEAL CAVITY.[5]

	Blood before experiment.		Recovered peritoneal fluid.					Blood			Urine.	
	Sodium chloride, per cent.	Δ	Cubic centimeters.	Sodium chloride, per cent.	Grams absorbed.	Δ	Refractive index.	Sodium chloride, per cent.	Δ	Dilution.	Cubic centimeters.	Sodium chloride, per cent.
Normal 0.85 per cent. sodium chloride, control.	.455	—0.60°	77	.53	.588	—0.593°	1.3353	.49	—0.586°	—.00133	11	.46
Normal 0.85 per cent. sodium chloride — adrenalin, intraperitoneal.		—0.62	41	.52	.811	—0.593	1.3354	.47	—0.586	—.00065	41	.53
Normal 0.85 per cent. sodium chloride — adrenalin, intravenous.			62.5	.58	.72	—0.593	—	.52	—0.593	—	11	.51
Normal distilled water, control.			44	.40	—	—0.57	1.3361	.41	—0.583	—.00136	14	.36
Normal distilled water — adrenalin, intraperitoneal.			21	.36	—	—0.553	1.3359	.41	—0.58	—.0012	49	.40
Normal 1.5 per cent. sodium chloride, control.			116	.59	1.1565	—0.634	1.3352	.56	—0.648	—.00026	8	.92
Normal 1.5 per cent. sodium chloride — adrenalin, intraperitoneal.			81	.62	1.2852	—0.654	1.3353	.51	—0.65	—.00035	30	.92

[5] The averages of the individual determinations are grouped together on the table which is added to this communication. Our experiments were made on 161 rabbits.

In animals that were injected with distilled water, instead of the 0.85 per cent. sodium chloride solution, we found in control animals that the freezing point of the blood serum at the end of the experiment was within the normal limits, averaging — 0.583°, and that the freezing point of the fluid recovered from the peritoneal cavity averaged slightly less, namely, — 0.57°. In animals into whose peritoneal cavity adrenalin was injected, in addition to the distilled water, the freezing point of the serum averaged — 0.58°; that of the peritoneal fluid, — 0.553°. Here the normal osmotic pressure of the blood was retained, in spite of the large amount of distilled water absorbed from the peritoneal cavity; and the osmotic pressure of the fluid in the peritoneal cavity was raised to almost that of the serum.

In control experiments, in which we injected 1.5 per cent. sodium chloride solution, we found the lowering of the freezing point of the serum to be increased to — 0.648°. When, in addition, adrenalin was injected, the freezing point was slightly lower, namely, — 0.656°. In the latter case the freezing point of the peritoneal fluid was also lower than in the experiments with 0.85 per cent. sodium chloride solution, averaging — 0.634° in the control experiments, and — 0.654° in the adrenalin experiments. Here the osmotic pressure of the serum appears to be slightly greater in the adrenalin experiments. The osmotic pressure of the peritoneal fluid was certainly greater in the adrenalin experiments. The molecular concentration of the blood serum was greater than that of the peritoneal fluids in both control and adrenalin experiments.

SODIUM CHLORIDE PERCENTAGES IN CONTROL ANIMALS.

In animals into whose peritoneal cavity 120 cubic centimeters of an 0.85 per cent. sodium chloride solution had been injected, we found an average of .588 gram of sodium chloride absorbed of a total of 1.02 grams injected. In these cases, the fluid recovered from the peritoneal cavity at the end of the experiment showed an average of .53 per cent. sodium chloride, while the blood serum showed an average of .49 per cent., and the urine, which in individual cases varied quite widely, showed .46 per cent. For every cubic centimeter of fluid absorbed, .014 gram of sodium chloride was absorbed.

SODIUM CHLORIDE PERCENTAGES IN ANIMALS INJECTED WITH ADRENALIN.

When, in addition to the 120 cubic centimeters of sodium chloride solution, adrenalin was injected intraperitoneally, we found that an average of .811 gram of sodium chloride was absorbed. The fluid recovered from the peritoneal cavity in such cases contained an average of .52 per cent. of sodium chloride; the blood serum, .47 per cent.; and the urine, .53 per cent. For every cubic centimeter of fluid absorbed, .010 gram of sodium chloride was removed from the peritoneal cavity.

In cases in which the adrenalin was injected intravenously, the absolute amount of sodium chloride absorbed from the peritoneal cavity was .72 gram. The sodium chloride content of the recovered fluid averaged .58 per cent.; of the blood serum, .52 per cent.; and of the urine, .51 per cent. Here .011 gram of sodium chloride was absorbed with every cubic centimeter of fluid.

Comparing these results with the results in control animals, we note that the adrenalin, whether injected intraperitoneally or intravenously, increases the absolute amount of sodium chloride absorbed from the peritoneal cavity. The sodium chloride percentage of the recovered fluid in control animals is slightly greater than in animals injected with adrenalin intraperitoneally. The percentage of the latter fluid is higher in the animals injected intravenously than in the controls, even though the actual amount of sodium chloride absorbed in such animals is greater than in the controls.

The sodium chloride content of the blood is slightly greater in control animals than in animals that received adrenalin intraperitoneally. In animals injected with adrenalin intravenously, the percentage of sodium chloride in the serum is higher than in the serum of the controls.

The sodium chloride percentage of the urine varied markedly in individual experiments. We find, however, the average in both the adrenalin series slightly higher than in the control series; so that in the course of the experiments in which adrenalin was administered, more sodium chloride must have been eliminated from the body than in the control experiments. The difference between the absolute quantities excreted by the control animals and those ex-

creted by the animals that had received adrenalin intravenously is slight; but between the control animals and the animals that had received the adrenalin intraperitoneally, the difference is very considerable.

SODIUM CHLORIDE PERCENTAGES IN ANIMALS INJECTED WITH DISTILLED WATER.

When, instead of the sodium chloride solution, distilled water was injected into the peritoneal cavity, we were able to determine the influence of adrenalin on the movement of salt into the peritoneal cavity. Thus in control animals into whose peritoneal cavity distilled water was injected, the average sodium chloride percentage of the recovered fluid was .40 per cent.; while the average sodium chloride percentage of the blood serum was .41 per cent.

In animals into which adrenalin was injected intraperitoneally, the sodium chloride percentage of the blood serum was the same as in the control series (.41 per cent.); but the average sodium chloride percentage of the recovered fluid was lower than that of the controls, being .36 per cent. It appears that sodium chloride passes more slowly from the blood into the peritoneal cavity when adrenalin is injected intraperitoneally. This finding corresponds with the results that Achard and Caillard obtained in guinea-pigs.

SODIUM CHLORIDE PERCENTAGES IN ANIMALS INJECTED WITH 1.5 PER CENT. SODIUM CHLORIDE SOLUTION.

In control experiments in animals into whose peritoneal cavity 1.5 per cent. sodium chloride solution was injected, we found the recovered fluid to contain an average of 0.59 per cent. sodium chloride. Thus, of 1.8 grams of sodium chloride injected, 1.1565 grams were absorbed, on the average. We found 0.55 per cent. of sodium chloride in the blood serum, and about 0.92 per cent. of sodium chloride in the urine.

In adrenalin experiments, the recovered fluid contained an average of 0.62 per cent. sodium chloride. Here 1.2852 grams of sodium chloride were absorbed. The blood serum contained 0.51 per cent. of sodium chloride; and the urine, 0.92 per cent. of the salt.

Thus, the injection of adrenalin has interfered with the relative absorption of sodium chloride, but increased the absolute absorption. There was less sodium chloride in the blood serum in the experiments with adrenalin. This may have been due to the increased amount of urine secreted in these experiments, thus freeing the body of the sodium chloride. In both series, the sodium chloride percentage in the blood was considerably higher than in experiments in which instead of the hypertonic solution 0.85 per cent. sodium chloride solution was injected intraperitoneally. The increase in sodium chloride excretion through the urine caused by the hypertonic solution did not seem to be influenced by the adrenalin. There was a slight difference between the average lowering of the freezing point of the recovered peritoneal fluid in the adrenalin and in the control series, this difference corresponding very closely to the difference in average sodium chloride percentages noted in these fluids.

DILUTION OF THE BLOOD IN ANIMALS INJECTED WITH 0.85 PER CENT. SODIUM CHLORIDE SOLUTION.

We also determined the degree of dilution of the blood as the result of the absorption of fluid from the peritoneal cavity. For this purpose, we determined the refractive index of the blood. The blood for the readings was taken at the beginning of the experiment—that is, before the injection of the fluid—and again, at the time the animal was killed. The readings were made with the Abbe Refractometer. The refractive index of the peritoneal fluid was also determined in a number of cases.

In the control animals into whose peritoneal cavity 0.85 per cent. of sodium chloride solution had been injected, the refractive index of the serum was reduced by 0.00133, on the average; while in animals in which adrenalin was injected intraperitoneally, the index was reduced by only 0.00065, the decrease in the refraction being about half as much as in the controls. This indicates that, under the influence of adrenalin, fluid leaves the blood vessels—an observation that is in agreement with the results obtained by Hess (7), Erb (8), and Böhm (9). They all agree in the fact that the blood is more concentrated after the injection of adrenalin.

The refractive index of the recovered peritoneal fluid averaged about the same in both cases (1.3354).

Thus, it appears that, in spite of a much increased absorption from the peritoneal cavity under the influence of adrenalin, less fluid is retained in the vascular system.

DILUTION OF THE BLOOD IN ANIMALS INJECTED WITH DISTILLED WATER.

In control animals into whose peritoneal cavity 120 cubic centimeters of distilled water were injected, we found the refractive index of the blood reduced, on an average by .00136; while in animals that were injected with adrenalin in addition to the distilled water, we found the average reduction of the refractive index to be .0012. Here the average reduction in the adrenalin animals was about as great as in the controls. In spite of the increased amount of fluid absorbed from the peritoneal cavity of rabbits injected with distilled water, the dilution of the blood was not increased in the latter animals, the dilution being practically the same in the sodium chloride solution and distilled water series.

In this series the refractive index of the peritoneal fluid averaged 1.3361 in the control animals, and 1.3359 in the adrenalin animals; thus, slightly more than in the animals that were injected with sodium chloride solutions. A relatively greater amount of proteid material remained in the peritoneal cavity in the experiments with distilled water.

DILUTION OF THE BLOOD IN ANIMALS INJECTED WITH 1.5 PER CENT. SODIUM CHLORIDE SOLUTION.

In these experiments the averages showed the refractive index of the blood serum to be lowered by 0.00026 in the control experiments, and by 0.00035 in the adrenalin experiments. From the results, it appears that the dilution of the blood was slight in both series; and possibly that there was a greater amount of water retained in the blood in the adrenalin experiments.

In the recovered peritoneal fluid, the refractive index averaged 1.3352 in the control experiments, and 1.3353 in the adrenalin experiments. These figures are slightly lower than in the experiments in which 0.85 per cent. sodium chloride solution was injected.

Discussion of Results.—Our experiments show conclusively that adrenalin increases the absorption of fluid from the peritoneal cavity and, at the same time, increases the amount of urine excreted. Is the absorption of fluid secondary to the diuresis? We believe that our experiments tend to prove that such a relation between absorption and diuresis does not exist. In the first place, after the intravenous injection of adrenalin, the amount of urine secreted was identical with the amount secreted in the control experiments; notwithstanding this fact, the absorption of fluid from the peritoneal cavity was increased in animals injected intravenously with adrenalin. Secondly, in individual experiments we did not find the relation between the amounts of fluid absorbed and the amounts of urine excreted to be very distinct. Thirdly, in experiments that we shall report more fully at some later date, we found that a substance that has a much more marked diuretic action than has adrenalin did not increase the absorption of fluid from the peritoneal cavity.

There does, however, appear to be a certain relation between the increased diuresis and increased absorption of fluid. In many of the individual experiments, we found that where more fluid was absorbed, more urine was excreted; although there cannot be said to be any direct relation between these two. Whether there exists an indirect relation between the absorption of fluid from the peritoneal cavity and the secretion of urine remains to be determined by further experiments.

Our experiments show that distilled water is more rapidly absorbed from the peritoneal cavity than an 0.85 per cent. sodium chloride solution, and that 0.85 per cent. sodium chloride solution is more rapidly absorbed than a 1.5 per cent. sodium chloride solution. These results are in conformity with previous experiments of Hamburger, Roth, Starling, O. Cohnheim and others. These facts suggested to us that adrenalin also may increase the absorption by increasing the molecular concentration of the blood.

In order to determine whether adrenalin, when injected intraperitoneally, changes the osmotic pressure of the blood, we injected normal rabbits with this substance every half-hour. These experiments, therefore, were carried out in exactly the same way as were

the absorption experiments, with the exception that no sodium chloride solution was injected into the rabbit. The animals were killed at various times after the third or fourth injection of adrenalin, and the blood was collected. One rabbit, killed very soon after the third injection, showed a lowering of the freezing point of — 0.67°. Another animal was killed fifteen minutes after the last injection; and in this animal, the lowering of the freezing point of the blood serum was — 0.62°. A rabbit killed twenty minutes after the last injection showed a lowering of the freezing point of — 0.625°. Half an hour after the last injection, the freezing point was — 0.605°. In two rabbits killed three quarters of an hour after the last injection, the freezing point was — 0.605° and — 0.57°.

Thus, it appears that adrenalin increases the osmotic pressure of the blood serum, when injected intraperitoneally. This increase is quite evident fifteen to twenty minutes after the injection of adrenalin, but gradually disappears; so that three quarters of an hour after the injection, the freezing point of the serum has almost returned to normal.

Thus the probability is strong that the repeated increases in the osmotic pressure of the serum caused by the repeated injections of adrenalin explains the increase in absorption of fluid.

It may be due to two causes that we did not note this greater lowering of the freezing point of the blood serum in the experiments in which we injected adrenalin intraperitoneally together with 0.85 per cent. sodium chloride solution: namely, the blood was usually not taken until half to three quarters of an hour after the last injection, when the effect had already largely disappeared; and, furthermore, it appears that the absorption of an 0.85 per cent. sodium chloride solution (which is slightly hypotonic as compared with the blood) will itself slightly lower the freezing point of the serum. Thus, while we found — 0.60° to be the lowering of the freezing point in normal rabbits, we found an average of — 0.586° in animals that had been injected with the sodium chloride solution.

A definite statement that the increased osmotic pressure of the blood is the sole factor that increases the absorption of fluid from the peritoneal cavity, when adrenalin is injected intraperitoneally

would not yet be warranted; but certainly this is the most important factor.

Of interest is the fact that the increased absorption of fluid from the peritoneal cavity takes place notwithstanding the contracted condition of the blood vessels prevailing under the influence of adrenalin, and that, in spite of the increased absorption of fluid, the dilution of the blood in animals injected with adrenalin is considerably less marked than in the control animals. That fluid passes from the peritoneal cavity into the blood vessels is apparent from the fact that in spite of the marked diuresis occurring in the adrenalin animals, we still found a small degree of dilution of the blood.

This lessened dilution of the blood cannot be due to the marked diuresis, since we found that the larger amount of fluid eliminated by the kidneys in the adrenalin experiments was compensated for by the increased amount of fluid absorbed from the peritoneal cavity. This effect of adrenalin is due to a specific action, causing more fluid to pass out of the vessels into the tissues of the body.

Does a relation exist between the rapidity of absorption and the size of the animal? Hamburger (10) has shown that when the intra-abdominal pressure is increased, the rate of absorption of fluid from the peritoneal cavity increases. If, however, the intra-abdominal pressure is increased above a certain limit, the rate is slowed. The differences beween the periods of time required for a fixed amount of fluid to be absorbed at the varying degrees of pressure are quite marked.

In our experiments, we injected relatively large quantities of fluid into the peritoneal cavity of rabbits of various sizes. Could the size of the rabbit have influenced the intra-abdominal pressure in our experiments, and thus have influenced the absorption? If such were the case, we should find the average amount of fluid absorbed in small animals to differ from the average amount absorbed in large animals. In order to determine the influence of the size of the animals upon absorption, we have arranged our experiments in two groups: large animals, weighing 1600 grams or more; and small animals, weighing under 1600 grams. The average weight of the group that includes the heavier animals is 1770 grams; and of the group that includes the smaller animals, it is 1220 grams.

In the control series into which 0.85 per cent. sodium chloride solution was injected, the average amount of fluid absorbed was exactly the same in both groups; and in the other series of experiments, the differences between the averages in the two groups were very slight. If, in our experiments, the size of the animal influenced the intra-peritoneal pressure, this influence was so slight as to be entirely negligible. The absorption, therefore, was not influenced by the variations in size of the animals used in our experiments.

It would, therefore, be incorrect to reduce the amount of fluid injected or absorbed to a certain unit of weight of the animal.[6]

We see from our results that it is not to be expected that a rabbit of twice the weight of another should absorb double the amount of fluid in a given time.

Concerning the influence of adrenalin on the absorption of sodium chloride, we found that in every case the absolute quantity of this salt absorbed from the peritoneal cavity was increased under the influence of adrenalin. There existed, however, certain differences in the relative absorption of sodium chloride in the different series.[7] When adrenalin was injected intraperitoneally, in combination with 0.85 per cent. sodium chloride solution, the relative sodium chloride absorption was slightly increased; when adrenalin was injected intravenously, the relative sodium chloride absorption was diminished. The slight increase in the relative absorption of sodium chloride after the intraperitoneal injection of adrenalin may have been due to the increased amount of sodium chloride excreted through the kidneys, causing a decrease in the amount of sodium chloride in the blood, which, in turn, would cause more sodium chloride to be taken up from the injected fluid. In experiments with the intravenous injection of adrenalin the apparent decrease in absorption was probably due to the increase in the amount of sodium chloride in the blood.

When 1.5 per cent. sodium chloride solution is injected, adrenalin seems to delay slightly the relative absorption of sodium chloride,

[6] This procedure was used, for instance, in the experiments of Meltzer and Salant (*Jour. of Med. Research,* 1903, ix, 33), in their study of the absorption in nephrectomized animals.

[7] By relative absorption of sodium chloride, we understand the absorption of sodium chloride as compared with that of the solution.

in spite of the fact that the sodium chloride percentage in the blood of such animals in our experiments was slightly less than in controls. This lower sodium chloride content of the blood was probably due to the increased elimination of sodium chloride through the kidneys.

When distilled water was injected intraperitoneally, the movement of sodium chloride into the peritoneal fluid was diminished more in the adrenalin experiments than in the controls. This may have been due to the increased excretion of sodium chloride in the urine, which would decrease the amount of sodium chloride in the blood; and thus, there would be less of this salt free to pass into the peritoneal fluid.

It seems, therefore, that although in experiments in which adrenalin was injected intravenously, and also in experiments in which, in addition to the 1.5 per cent. sodium chloride solution, adrenalin was injected intraperitoneally, the absolute amount of sodium chloride absorbed was increased, the relative amount of sodium chloride in the fluid remaining in the peritoneal cavity was somewhat greater than in controls. To what this effect of adrenalin is due, we cannot state; but it appears not to be of very great importance, since in experiments in which, in addition to 0.85 per cent. sodium chloride solution, adrenalin was injected *intraperitoneally,* we found the relative absorption of sodium chloride to be increased. It may be noted that we found the sodium chloride percentage of the blood slightly higher after the absorption of 0.85 per cent. sodium chloride solution than it was in normal animals. The average sodium chloride content of the blood serum of six normal rabbits was .45 to .46 per cent.; while in control experiments we found .49 per cent., and in adrenalin experiments, .47 per cent. As was to be expected, after an injection of 1.5 per cent. sodium chloride solution, we found the sodium chloride content of the blood even more increased in both control and adrenalin experiments. The absorption of distilled water, on the other hand, decreased the sodium content of the blood serum in both control and adrenalin experiments.

The movement of osmotically active substances other than sodium chloride into or from the peritoneal cavity does not appear to have been much influenced by the intraperitoneal injection of adrenalin.

After the intraperitoneal injection of 0.85 per cent. sodium

chloride solution and adrenalin, the sodium chloride percentages and freezing points of the recovered peritoneal fluids were approximately the same as in the controls in which no adrenalin had been administered. In such cases, the osmotic pressure of the blood serum was also the same as in the blood serum of control experiments, in spite of the fact that its sodium chloride content was less in the adrenalin experiments.

In the experiments in which 1.5 per cent. sodium chloride solution was injected intraperitoneally, the greater sodium chloride percentage in the peritoneal fluid noted in the adrenalin experiments was associated with the higher osmotic pressure. In the blood serum, the osmotic pressure was likewise greater in the adrenalin experiments than in the controls; but the sodium chloride content of the blood serum was less in the adrenalin experiments.

When distilled water was injected intraperitoneally, we found in the adrenalin experiments a lower osmotic pressure of the peritoneal fluid, associated with a lower sodium chloride content. In the blood serum, the sodium chloride percentages were approximately the same in control and in adrenalin experiments, but the osmotic pressure of the blood serum in adrenalin experiments was slightly lower than in control experiments.

It appears, therefore, that in these experiments there exists a certain parallelism between the osmotic pressure of the recovered peritoneal fluid and the sodium chloride content in each series. The amounts of osmotically active substances other than sodium chloride present in the peritoneal fluid must therefore have been approximately the same in the adrenalin and in the control experiments.

When, however, adrenalin was injected intravenously we found that, while the osmotic pressure of the recovered peritoneal fluid was the same as in the control experiments, the sodium chloride content of the recovered fluid was relatively greater. In the blood serum of such animals, we found both the osmotic pressure and the sodium chloride percentages higher than in the control experiments. In these experiments there was therefore a relatively smaller quantity of osmotically active substances other than sodium chloride present in the peritoneal fluid than in control experiments.

We found, both in experiments with the intravenous injection of adrenalin and in those with the injection of 1.5 per cent. of sodium chloride solution and adrenalin, that under the influence of adrenalin the osmotic pressure of the blood serum was higher than in the corresponding control experiments. In the former, this was accompanied by an increased sodium chloride percentage in the blood; in the latter, by a lower sodium chloride percentage. The sodium chloride percentage of the peritoneal fluid was higher than in controls in both of these sets of experiments; the osmotic pressure of the peritoneal fluid was higher than that of the blood serum in experiments with 1.5 per cent. sodium chloride solution and adrenalin, and the same as that of the serum in experiments in which adrenalin was injected intravenously.

It thus appears that, under certain conditions, adrenalin inhibits the normal movements of sodium chloride and other osmotically active substances to or from the blood or the peritoneal cavity. Thus, although in both these cases (mentioned above) we found relatively less absorption of sodium chloride from the peritoneal cavity, we found an increased osmotic pressure of the blood, which in one case was entirely or partly due to the increase in chlorides, but which in the other case was not due to an increase in chlorides; so that in the latter case, other osmotically active substances must have been drawn from the tissues into the blood, in order to maintain this higher osmotic pressure. Under these conditions, adrenalin maintained an increase in the osmotic pressure of the blood, just as it did in control experiments in which adrenalin was injected without an additional intraperitoneal injection of some fluid.

In experiments in which 0.85 per cent. sodium chloride solution was injected intraperitoneally, we found, both in controls and in those in which adrenalin was injected intraperitoneally, that the osmotic pressure of the peritoneal fluid was slightly higher than that of the blood serum, in spite of the fact that the injected salt solution was somewhat hypotonic as compared with the blood serum. When adrenalin was injected intravenously, the osmotic pressure of the recovered peritoneal fluid was the same as that of the blood serum.

There existed, therefore, after the intraperitoneal injection of

0.85 per cent. sodium chloride solution, and also when adrenalin was added intraperitoneally, a tendency of the intraperitoneal fluid to attain a greater osmotic pressure than that of the blood serum. This is of especial interest, because we note a similar condition in cases of general edema in man, in which the osmotic pressure of the ascitic fluid surpasses that of the other edematous fluids and also that of the blood serum. Thus there exists, normally, a mechanism that causes the passage of osmotically active substances from the blood or the tissues into the peritoneal cavity to such an extent that the osmotic pressure of the peritoneal fluid surpasses that of the blood; and it follows from our experiment that such a mechanism is not dependent upon pathological changes, inasmuch as we found it apparent even in normal animals that had received an intraperitoneal injection of a slightly hypotonic salt solution.

When distilled water was injected into the peritoneal cavity, we found, as might have been expected, that the osmotic pressure of the recovered peritoneal fluid in both control and adrenalin experiments was lower than that of the blood serum. On the other hand, when hypertonic sodium chloride solutions were injected, the osmotic pressure of the blood serum had risen and was greater in both adrenalin and control animals at the end of the experiments than was the osmotic pressure of the peritoneal fluid.

The movement of albuminous material does not seem to have been much influenced by adrenalin. However, the various solutions injected changed somewhat the amounts of albuminous material found in the peritoneal fluid. In those experiments in which the smallest amount of fluid was recovered (namely, the experiments in which distilled water was injected), we found relatively the greatest amount of albuminous matter. In experiments in which the largest amounts of fluid were recovered (namely, when 1.5 per cent. sodium chloride solution was injected), we found relatively the smallest amount of proteid matter in the peritoneal fluid. In the experiments in which 0.85 per cent. sodium chloride solution was injected intraperitoneally, and in which the amounts of fluid recovered stood midway between the amount recovered in the experiments in which distilled water and those in which 1.5 per cent. sodium chloride solution were used, we found in the recovered peri-

toneal fluid a relative amount of albuminous matter that stood midway between the amounts found in these two former series.

We have found that adrenalin distinctly increases the absorption of fluid from the peritoneal cavity. Therefore, the influence of adrenalin in increasing the amount of peritoneal transudate, when large amounts of 0.85 per cent. sodium chloride solution, to which adrenalin had been added, were infused into rabbits, must have been due to an increase in the transudation into the peritoneal cavity. Indeed, this increase in transudation must have been far greater than is shown by comparisons of the amounts of peritoneal transudate found in the sodium chloride and the adrenalin-sodium chloride experiments; for in the latter, as the result of the increased absorption, a greater amount of fluid is constantly being withdrawn from the peritoneal cavity.

CONCLUSIONS.

1. Adrenalin injected intraperitoneally increases the rapidity of absorption of fluid from the peritoneal cavity, independently of whether the solution to be absorbed is hypotonic or hypertonic or is approximately isotonic with the blood serum. The intravenous injection of adrenalin also increases the absorption of fluid, but not so markedly as does the intraperitoneal injection.

2. Adrenalin injected either intraperitoneally or intravenously increases the quantity of sodium chloride absorbed. The relative absorption of sodium chloride—the movement from the peritoneal cavity of sodium chloride, as compared with the movement of water—is slightly increased when 0.85 per cent. of sodium chloride solution and adrenalin are injected intraperitoneally; but it is diminished when adrenalin is injected intravenously, or when 1.5 per cent. sodium chloride solution and adrenalin are injected. When distilled water has been injected intraperitoneally, adrenalin decreases the relative amount of sodium chloride in the peritoneal fluid—a fact that is evidently related to the increased elimination of sodium chloride through the kidneys under the influence of adrenalin.

3. When 0.85 per cent. sodium chloride solution is injected into the peritoneal cavity, the blood becomes diluted after two hours

and a half. When adrenalin is also injected, the dilution of the blood is less marked, in spite of the increased absorption under the influence of adrenalin. When distilled water is injected into the peritoneal cavity, the blood is diluted equally in control and adrenalin experiments. When 1.5 per cent. sodium chloride solution is injected, the dilution of the blood is very slight, and in adrenalin experiments it is the same as in control experiments or very slightly greater than in control experiments.

4. The increase of absorption from the peritoneal cavity caused by the injection of adrenalin is not due to the increased diuresis caused by the injection of this substance.

5. The injection of adrenalin causes a temporary increase in the osmotic pressure of the blood, which gradually returns to normal. Under certain conditions, after the injection of adrenalin, there is a tendency toward maintaining the higher osmotic pressure of the blood serum, even up to the end of the experiment. We have reason to believe that this increase in the osmotic pressure of the blood is the main factor in increasing the absorption of fluid from the peritoneal cavity.

6. In experiments in which 0.85 per cent. sodium chloride solution has been injected intraperitoneally, either with or without adrenalin, there exists a tendency of the peritoneal fluid to attain a greater osmotic pressure than the blood serum, in spite of the fact that the injected fluid is slightly hypotonic as compared with the blood serum. We note a similar condition in cases of general edema in man, in which the osmotic pressure of the ascitic fluid is greater than that of the other edematous fluids, or even that of the blood serum. There exists, therefore, a mechanism that causes the passage of osmotically active substances from the blood or from the tissues into the peritoneal cavity, and that causes the osmotic pressure of the peritoneal fluid to become higher than that of the blood. It follows from our experiments that this mechanism, which causes the ascites in edematous persons to have such a high osmotic pressure, is not dependent upon certain pathological changes in the lining membranes or upon other pathological conditions, but exists already in normal animals.

7. The addition of 1.22 per cent. calcium chloride solution to

0.85 per cent. sodium chloride solution, in such proportions as we used in our infusion experiments, in which we determined the transudation into the peritoneal cavity, delays the absorption of fluid from the peritoneal cavity but very slightly. Therefore, calcium chloride increases directly the transudation into the peritoneal cavity and does not cause an increase in the amount of fluid in the peritoneal cavity merely by inhibiting the absorption.

8. It follows that adrenalin does not increase the amount of peritoneal transudate found after the intravenous infusion of large quantities of sodium chloride solution, to which adrenalin has been added, by delaying the absorption from the peritoneal cavity. The increased amounts of peritoneal fluid must be due to increased transudation into the peritoneal cavity; and the adrenalin, in view of its marked effect on absorption from the peritoneal cavity, must increase the movement of fluid into the peritoneal cavity much more strongly than could be assumed from the figures obtained in the infusion experiments.

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OBSERVATIONS AND EXPERIMENTS ON THE SO-CALLED THYROID CARCINOMA OF BROOK TROUT (SALVELINUS FONTINALIS) AND ITS RELATION TO ORDINARY GOITRE.[1]

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J. Exp. Med. 12. 30, 1910

Plates XXV and XXVI.

INTRODUCTION.

This disease is characterized by the abnormal and excessive growth of thyroid tissue leading to the formation of visible tumors which may appear ventrally in the region below the base of the tongue or dorsally in the floor of the mouth and pharynx between the first and third gill arches. Secondary growths (?) are frequently to be seen in the gills and at the anterior extremity of the lower jaw (see Plate XXV, Fig. 1).

So far as is definitely known at present this extreme degree of the disease is confined to carnivorous fish reared in captivity. Its spread has been parallel with the recent great development of fish culture and the artificial rearing of large numbers of game fish—especially *trout* and *salmon*.

According to Pick[2] this disease was first positively recognized as carcinoma of the thyroid by Marianne Plehn.[3] The disease had been definitely known since the seventies, but its exact nature or even

[1] Read before the Cleveland Academy of Medicine, February 11, 1910. Received for publication February 8, 1910.

[2] *Berliner klin. Woch.*, 1905, xlii, 1435.

[3] *Allg. Fischerei Zeitung*, 1902, p. 117.

its thyroid origin was disputed.[4] At present the disease prevails in all countries where trout and salmon are artificially reared.

Up to the present time no systematic studies have been recorded apart from the histological observations above referred to. The observations and experiments here reported were undertaken to ascertain whether there was any relation between ordinary goitre (active thyroid hyperplasia) as seen in fish and animals, and this so-called cancerous affection of the thyroid. Pick, from his purely anatomical studies, has suggested that this cancerous affection might be only the terminal stage of an earlier simple goitrous condition.

GENERAL OBSERVATIONS.

Our observations and experiments were made during October and November, 1909, at a large private fish hatchery in the mountains of Pennsylvania.

The arrangement of the houses and retaining tanks is quite simple. This is shown in Fig. 1 (see page 314). The tanks[5] are arranged in a single series down the course of the brook. The water supply is likewise simple. There is but a single spring supplying the upper six houses while the lower five receive the water that has passed through the upper six together with that from a second small spring and a six inch pipe line from a large stream about a quarter of a mile to the left. On account of the increased water supply, and for the clearer understanding of certain observations to be mentioned later, it is best to divide the series of tanks into an upper and a lower division. Between the upper and lower divisions of houses (that is between Houses VI and VII) the water follows the original brook for a distance of about a quarter of a mile. Then it is again collected by means of a dam and enters the lower division of houses with the additions to its volume above mentioned. The water coming from the spring above all houses to

[4] Pick, *loc. cit.*

[5] We shall use the word "tank" throughout this paper although they are usually designated "ponds" by fish culturists. These tanks are made by excavating the soil and shoring up their walls either with plank or concrete. The inclosed space may be of any dimensions desired, but at the hatchery in question they averaged six to seven feet wide by twelve to sixteen feet long and two to three feet deep. The bottoms are sometimes made of plank, sometimes of concrete but more commonly of sand or gravel.

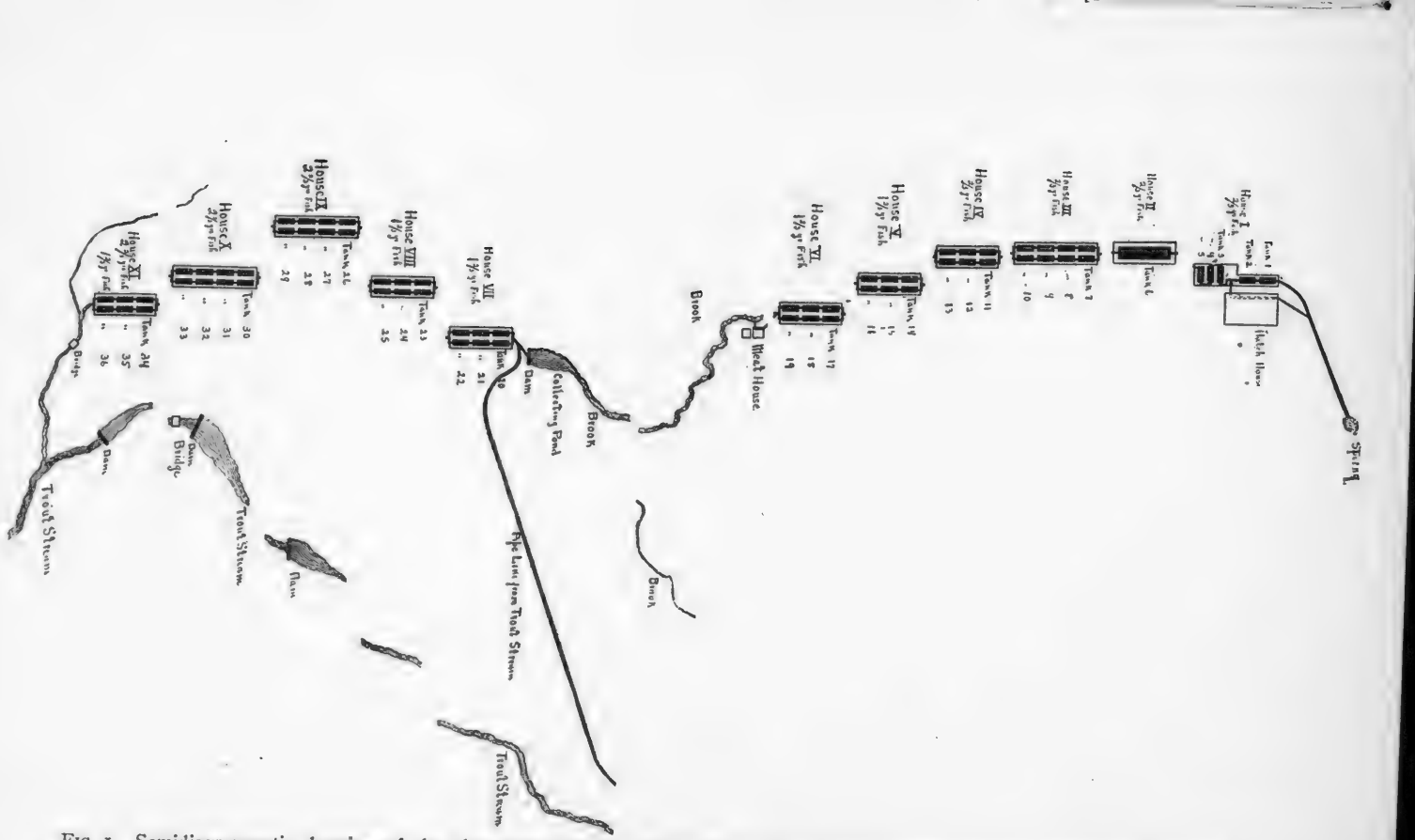


FIG. 1. Semidiagrammatic drawing of the plan, arrangements and location of the hatchery, water supplies, houses and retaining tanks.

the first house—a distance of about fifty yards—is also allowed to follow its natural bed. Apart from these two exceptions, the water is carried in covered raceways made of lumber.

The distribution of the fish according to *age* is important and, accidentally, fortunate from our point of view, inasmuch as the three pairs of tanks likely to show the greatest variations in the condition of the thyroids contained fish of the same age, namely one and two-thirds years.

Taking up the arrangement according to age (see Fig. 1) it is seen that Houses I, II, III and IV contain eight months old fish; Houses V, VI, VII and VIII contain one and two-thirds years old fish, and Houses IX, X and the first two pairs of tanks in House XI contain two and two-thirds years old fish. The last pair of tanks in House XI contains one and two-thirds years old fish. As already pointed out, the three most important locations as regards the effect of water supply and water pollution are Houses VI, VII and the last pair of tanks of House XI, which contain fish of the same age, that is, one and two-thirds years.

It occurred to us that it might be of importance to ascertain the number of fish with visible tumors in a given number of fish at these locations. Accordingly, we examined series of two hundred and ten fish from each of these locations, the last pair of tanks in House XI, the uppermost pair of tanks in House VII and the last pair of tanks in House VI. The fish were collected in a seine and two hundred and ten counted without selection on two successive days.

TABLE I.

House XI	Tank 36	210 fish	3 visible tumors.
House XI	Tank 36	210 fish	4 visible tumors.
House VII	Tank 20	210 fish	2 visible tumors.
House VII	Tank 20	210 fish	3 visible tumors.
House VI	Tank 19	210 fish	6 visible tumors.
House VI	Tank 19	210 fish	8 visible tumors.

The result was surprising in that the uppermost tank (19) shows a much larger percentage of visible tumors. Tanks 36 and 20 are practically the same, the percentage being perhaps slightly less in Tank 20. To explain the great differences between Tanks 19 and

20, the possibility of one tank having a larger number of fish was raised, but the caretaker told us that the same number of fish was placed in each. From our examination we should agree that there was no very great difference in the number in the two tanks. A more important and direct factor lies in the water supply and its pollution. Tank 20 receives about three times the amount of water that Tank 19 does, and it has the advantages that the two additional supplies are fresh from the soil, and also that the water from Tank 19 has traversed its natural brook bed for a distance of nearly a quarter of a mile before again entering the tanks. Thus as regards volume and pollution of the water, Tank 19 is in the worst position. We are inclined to attach considerable importance to this decreased water supply and increased pollution as explaining the more severe thyroid disease in Tank 19, and also the increased water supply with its factors of dilution and perhaps some reduction in the products of decomposition of food and excreta as explaining the greatly improved state of the fish in Tank 20.

This same difference is distinctly noticeable in the fish without visible tumor, as will be referred to in the discussion of the "tank series" which follows.

OBSERVATIONS ON THE HISTOLOGICAL STATE OF THE THYROID GLANDS IN A SERIES OF FISH REPRESENTING SPECIMENS FROM ALL TANKS

Following up the lead gained from enumerating the number of visible tumors in a given series of fish of the same age from crucial points in the line of tanks we collected specimens without choice from all the tanks, also specimens of different ages from the raceways *above* and *below* all houses, and lastly, specimens of the larger three and two-third years old fish from the trout stream near by.[6]

We have tabulated the histological condition of the thyroids and have arranged them in order from above downward (Table II).

[6] The plan followed at the club has been that each April the three year old fish are transferred to the trout stream whence they are taken by the club members with hook and line.

TABLE II. *Tank (Pond) Series.*

House No.	Tank (pond) No.	Age of fish in years.	Histological condition of thyroid gland.			Classification.
			Stainable colloid.	Distribution, infiltration, etc.	Epithelium.	
Normal from brook above all houses, male.		2⅔	Normal.	Scattered groups of follicles about aorta and isolated follicles throughout areolar tissue.	Regular flat cubical.	Normal thyroid.
Normal from brook above all houses, male.		1⅔	"	Scattered groups of follicles about aorta and isolated follicles throughout areolar tissue.	" " "	" "
I	1	⅔	Absent.	Entire thyroid area filled, invasion of bone and muscle.	Uniform high columnar.	Marked glandular hyperplasia.
I	2	⅔	Nearly absent.	Entire thyroid area filled, invasion of bone and muscle with extensions toward gill segments.	" " "	" " "
I	3	⅔	Present, reduced.	Entire space about aorta filled with thyroid tissue, bone and muscle not involved.	" columnar.	Early moderate glandular hyperplasia.
I	4	⅔	Reduced.	All structures beneath pharyngeal mucosa including bone and muscle involved.	" "	Moderate glandular hyperplasia.
I	5	⅔	Nearly absent.	All structures beneath pharyngeal mucosa including bone and muscle involved.	" "	" " "
II	6	⅔	" "	Entire areolar space about aorta filled.	" "	" " "
III	7	⅔	Absent.	Throughout all structures beneath pharyngeal mucosa, infiltration and atrophy of bone, muscle, etc.	" high columnar.	Marked glandular hyperplasia.
III	8	⅔	Reduced.	Entire areolar space about aorta filled.	" columnar.	Moderate " "
III	9	⅔	None.	Entire space beneath pharyngeal mucosa occupied, destruction of bone and muscle.	" high columnar.	Marked " "
III	10	⅔	"	Entire space beneath pharyngeal mucosa occupied, destruction of bone and muscle.	" " "	" " "

TABLE II. *Tank (Pond) Series.* (Continued.)

House No.	Tank (pond) No.	Age of fish in years.	Histological condition of thyroid gland.			Classification.
			Stainable colloid.	Distribution, infiltration, etc.	Epithelium.	
IV	11	2/3	Nearly absent.	Entire space beneath pharyngeal mucosa occupied, destruction of bone and muscle.	Uniform high columnar.	Marked glandular hyperplasia.
IV	12	2/3	Absent.	Entire space beneath pharyngeal mucosa occupied, destruction of bone and musle, extension into gills.	" " "	" " "
IV	13	2/3	"	Entire space beneath pharyngeal mucosa occupied, destruction of bone and muscle, extension into gills.	" " "	" " "
V	14	1 2/3	"	Entire subpharyngeal space filled, cartilage, bone and muscle destroyed, extension into gills.	" " "	" " "
V	15	1 2/3	"	Entire subpharyngeal space filled, cartilage, bone and muscle destroyed, extension into gills.	" " "	" " "
V	16	1 2/3	"	Entire subpharyngeal space filled, cartilage, bone and muscle destroyed, extension into gills.	" " "	" " "
VI	17	1 2/3	"	Entire subpharyngeal space filled, cartilage, bone and muscle destroyed, extension into gills, with extensive fibrosis.	" " "	" " "
VI	18	1 2/3	"	Entire space beneath pharyngeal mucosa filled, bone, cartilage and muscle eroded.	" " "	" " "
VI	19	1 2/3	"	Entire space beneath pharyngeal mucosa filled, bone, cartilage and muscle eroded.	" " "	" " "
VII	20	1 2/3	Reduced, but everywhere present in the follicles.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	Uniform columnar.	Colloid—moderate marked glandular hyperplasia, involuting (reverting).

TABLE II. *Tank (Pond) Series.* (Continued).

House No.	Tank (pond) No.	Age of fish in years.	Histological condition of thyroid gland.			Classification.
			Stainable colloid.	Distribution, infiltration, etc.	Epithelium.	
VII	21	1⅔	Nearly normal.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	Uniform high cubical.	Colloid—early glandular hyperplasia, involuting (reverting).
VII	22	1⅔	Present throughout—reduced.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	" columnar.	Colloid—moderate glandular hyperplasia, involuting (reverting).
VIII	23	1⅔	Present throughout—reduced.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	" "	Colloid—moderate glandular hyperplasia, involuting (reverting).
VIII	24	1⅔	Present throughout—reduced.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	" "	Colloid—moderate glandular hyperplasia, involuting (reverting).
VIII	25	1⅔	Present throughout—reduced.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	" "	Colloid—moderate glandular hyperplasia, involuting (reverting).
IX	26	2⅔	Absent.	Every structure beneath pharyngeal mucosa infiltrated, extensions into gills.	High columnar, slightly irregular.	Marked glandular hyperplasia.
IX	27	2⅔	"	Every structure beneath pharyngeal mucosa infiltrated, extensions into gills.	High columnar, slightly irregular.	" " "
IX	28	2⅔	Present—reduced.	Every structure beneath pharyngeal mucosa infiltrated, extensions into gills.	High columnar.	" " "
IX	29	2⅔	Absent.	Every structure beneath pharyngeal mucosa infiltrated, extensions into gills, follicles in many places undifferentiated.	Irregular columnar.	Marked glandular hyperplasia, with extreme generalized fibrosis of thyroid area.
X	30	2⅔	Reduced.	Every structure beneath pharyngeal mucosa infiltrated, extensions into gills, follicles in many places undifferentiated.	" "	Colloid—marked glandular hyperplasia, with extensive generalized fibrosis of thyroid area.

TABLE II. *Tank (Pond) Series.* (Continued).

House No.	Tank (pond) No.	Age of fish in years.	Histological condition of thyroid gland.			Classification.
			Stainable colloid.	Distribution, infiltration, etc.	Epithelium.	
X	31	2⅔	Reduced.	All structures invaded including extension into gills.	Irregular columnar.	Colloid—marked glandular hyperplasia, with extensive generalized fibrosis of thyroid area.
X	32	2⅔	"	All structures invaded including extension into gills.	" "	Colloid—moderate glandular hyperplasia, extreme general fibrosis.
X	33	2⅔	Everywhere present.	All structures invaded including extension into gills.	" "	Colloid—moderate glandular hyperplasia, generalized fibrosis of thyroid area.
XI	34	2⅔	Reduced.	All structures invaded including extension into gills.	Columnar.	Colloid—marked glandular hyperplasia, extensive generalized fibrosis of thyroid area.
XI	35	2⅔	"	All structures invaded including extension into gills.	High columnar.	Marked glandular hyperplasia, slight general fibrosis.
XI	36	1⅔	"	All subpharyngeal structures involved, including bone, muscle and extensions to gills.	" "	Marked glandular hyperplasia.
Female from raceway BELOW all houses.		3⅔	Normal.	All subpharyngeal structures involved, including bone, muscle and extensions into gills.	Low cubical.	Pure colloid goitre (transferred from tank to brook as a three-year old in April,'09), spontaneous recovery.
From raceway BELOW all houses.		1⅔	"	All tissues beneath pharyngeal mucosa infiltrated, including bone and muscle.	Flat cubical.	Pure colloid goitre (transferred from tank to brook as a yearling in spring,'09), spontaneous recovery
From raceway BELOW all houses.		⅔	Present.	Perhaps a very slight increase in the number of thyroid follicles, no invasion of bone or muscle.	Cubical.	Normal (normal—early glandular hyperplasia)?
From trout stream.		3⅔	Normal.	All structures beneath pharyngeal mucosa infiltrated, bone, muscle and extensions to gills.	Flat cubical.	Pure colloid goitre (this is an example of the three-year old fish transferred from tanks to trout stream in April,'09), spontaneous recovery.

Beginning with the fish taken from the raceway above all houses, the thyroids were found to be normal in each instance irrespective of the age of the fish (Fig. 1). These fish living in the raceway, we were told, had spent their entire lives, since removal from the troughs as fry, in this small stream of water of about forty yards in length. They were not fed or cared for in any way.

Passing on to the tanks, it is seen that Tanks 1 to 13 contain two-thirds year old fish, that is, fish hatched in the winter of 1909. They were all healthy large fish. Histological examination revealed the fact that all these fish had marked thyroid changes although none had visible ventral tumors. This thyroid hyperplasia had in most cases invaded the striped muscle and bone and filled in all the areolar tissues beneath the epithelium of the floor of the pharynx. There was very little difference in the extent of the thyroid proliferation throughout the 13 tanks, although the specimens taken from Tanks 10 to 13 showed the most marked proliferation.

The one and two-thirds years old fish are contained in Tanks 14 to 25 inclusive. Here very great differences are to be noted in the thyroid condition between the fish from Houses V and VI of the upper division and Houses VII and VIII of the lower division. As pointed out above, the number of visible tumors was very much greater in the upper houses (V and VI) than in the lower houses (VII and VIII). So also the histological appearance of the thyroids is very much worse in Houses V and VI than in VII and VIII. In the fish of the upper houses the epithelial proliferation has extended to all the structures beneath the floor of the pharynx and even out into the gill arches. There is rarely any stainable colloid to be seen and the epithelium is everywhere columnar. In other words, the epithelial proliferation, started during their first year of life, has progressively increased during this their second year of life. On the other hand, the thyroids of fish from Houses VII and VIII contain moderate amounts of stainable colloid. The epithelium is, in general, low columnar, and the extent of the proliferation has not progressed beyond the stage reached during the first year of life while occupying Tanks 1 to 13. In other words, some agency has been acting which has successfully prevented further hyperplasia and even started the process of involution (reversion) of the thyroid hyperplasia to the colloid state. As above mentioned, the most patent factor seems to be the greatly increased water supply with its added factors of increased purity and dilution of the products of decomposition and putrefaction.

Passing now to the two and two-thirds years old fish, it is seen that they occupy Tanks 26 to 35 inclusive. In all of these fish the thyroids have undergone very extensive changes and added factors of histological complexity have to be taken into consideration by reason of their longer lives; the fact that they have been moved twice (that is, occupying Tanks 1 to 13 during their first year and Tanks 14 to 25 during their second year); the fact that it is impossible to determine which of the upper tanks they had previously occupied—all these factors undoubtedly enter into the explanation and interpretation of the more varied histological appearance of the glands, just as similar factors are known to modify mammalian thyroid hyperplasias. In most of the specimens examined there is stainable colloid present. The extent of the proliferation and the epithelial types are quite variable. In general, the epithelium is of the columnar

type as opposed to the high columnar type noted in Houses I to VI. Another factor of perhaps great importance is the noteworthy increase in the fibrous stroma, both relative and absolute, throughout the area of thyroid invasion. While it is impossible to speak of this group in terms specifically applicable to all the specimens, the general impression gained is that the proliferation is less active and the tendency toward the arrest of the growth is more evident. The prominence of the stroma, the presence of the colloid and the lessened height of the epithelium are, we believe, evidences of this tendency toward arrest.

Next in order, several specimens taken from the tail-race below all houses were examined. Here, as in the raceway above all houses, the fish are left to care for themselves. Several specimens of two-thirds year old fish were taken. These fish, the caretaker told us, were in all probability specimens of the winter hatch which could not be taken care of in the tanks and were planted in the trout stream and had come into the tail-race to avoid the bigger fish of the larger stream. The thyroids of these fish were slightly larger than in normal fish of the same age. Their colloid contents and epithelium were normal. We took this to mean that even though living in the polluted water below all the houses, but in the wild state, they were able to recover from whatever active thyroid hyperplasia they had at the time of planting, and also to maintain normal types of thyroid while living in such environment. We also took specimens of three and two-thirds years old fish both from the tail-race (fish that had come up from the larger stream to spawn) and from the main stream above the entrance of the tail-race stream. In these specimens we observed, perhaps, the most important changes thus far recorded, namely, that in all these fish the thyroids had returned to the pure colloid state, although the entire sub-pharyngeal space was filled with thyroid follicles including the bone, cartilage and striped muscle. Here one sees written plainly the life history of these fish thyroids, that is, that the extreme thyroid proliferation and invasion of all the structures beneath the pharyngeal mucosa occurred during their stay in the tanks, and that during their five to six months' stay in the open stream they had completely recovered from the disease and their thyroids had returned to as nearly their normal state as was possible for such hyperplastic thyroids.

To sum up, it has been seen that the fish which lived in the raceway above all houses and which had never been confined in the tanks maintain normal thyroids throughout their lives. Then, beginning with the series of tanks, it was shown that the eight months old fish are markedly affected even in the uppermost of all the tanks and that there is a gradual increase in the degree of thyroid proliferation which reaches its greatest extent and severity in the last house (VI) of the upper division. Beginning with House VII there is a marked improvement and lessening of the active thyroid proliferation coincident with the greatly increased water supply and the probable purification of the water from the upper division in its passage for a quarter of a mile along the original bed of the brook.

This improvement cannot be followed definitely throughout the remaining houses of the lower division for the reason that the two and two-thirds years old fish are housed here, but the general impression is that this improvement is maintained throughout the remaining tanks to a gradually lessened extent. Lastly, the examination of fish from the tail-race, living wild, but in the polluted water, has shown that their thyroids are normal in type. Also examination of older fish obtained from both the tail-race and the larger stream above the entrance of the tail-race into it, have shown that although the thyroid tissue has invaded all the structures beneath the pharyngeal mucosa at some past period in their lives, nevertheless, their stay of five to six months in a natural environment has effected a complete natural relief from the exciting cause or causes of the thyroid proliferation and that the thyroids have assumed their resting or colloid state.

EFFECT OF IODIN ON THE THYROID HYPERPLASIA.

This experiment was carried out as follows.

An ordinary trough with inside measurements of 120 by 20 by 18 inches, and a capacity of twenty-five cubic feet, was placed in House XI and arranged so that water could be supplied at a uniform rate from the main stream supplying the tanks. The water supply was regulated by means of a shunt and it was so arranged that in the fall of ten inches the column was broken to allow aeration. This stream was adjusted so that it filled the trough in four hours, thus making it possible for the complete replacement of the water six times every twenty-four hours. This rate of flow was kept the same throughout the experiment.

On October 9, six healthy-looking and clinically tumor-free fish were taken from House XI and Tank 36, killed at once and kept for *controls*. At the same time, fifteen healthy-looking and clinically tumor-free fish were taken from the same tank and placed in the trough. Iodin in the form of Lugol's solution (KI = 3 grams, I = 1 gram, water = 300 cubic centimeters) was used. Two cubic centimeters of this solution were added to the water of the trough each morning and evening. One fish was taken every second day for histological examination (October 11, 13, 15, etc.). The result of the histological examinations follows.

The six specimens (Table III) taken as controls show quite uniform thyroid changes. In all, the thyroid proliferation extended into the bone and the striped muscle and occupied the entire fatty areolar space beneath the floor of the pharynx The colloid was reduced in all and was practically absent in two. The epithelium was columnar in all and the degree of proliferation ranged between *moderate* and *marked* glandular hyperplasia—using the same standards as adopted for the mammalian thyroid hyperplasias.[1]

[1] The fish used in the inoculation experiment were the actual controls for the iodin experiment, inasmuch as all factors were the same for each experiment

TABLE III

Control Fish. Iodin Experiment.

No. of fish.	Age in years.	Histological condition of thyroids.			Classification.
		Colloid.	Infiltration.	Epithelium.	
1	1⅔	Reduced	Bone, muscle and all the areolar tissues beneath the pharyngeal mucosa	Columnar	Moderate glandular hyperplasia
2	1⅔	Reduced	Bone, muscle and areolar tissues	High columnar	Moderate glandular hyperplasia
3	1⅔	Practically absent	Extensive growth in bone, muscle cartilage and all the areolar tissues beneath pharyngeal mucosa	High columnar	Marked glandular hyperplasia
4	1⅔	Reduced	General growth throughout areolar tissue. No definite invasion of bone and muscle	Columnar	Moderate glandular hyperplasia
5	1⅔	Reduced	Bone, muscle and areolar tissues beneath pharyngeal mucosa	Columnar	Moderate glandular hyperplasia
6	1⅔	Practically absent	Bone, muscle, cartilage and all areolar tissues	High columnar	Moderate marked glandular hyperplasia

The fifteen specimens recorded in Table IV had been exposed to the iodin-containing water for periods ranging from two to twenty-two days. The experiment was terminated on the twenty-second day and the remaining five specimens taken.

It can be seen that little or no change is to be noted until the eighth to the tenth day. Then the increase of colloid and the changes in the epithelium toward cubical are evident. The process is gradual, reaching the pure colloid state on the twentieth day in one case, although, in two of the specimens taken on the twenty-second day, the return to colloid was not quite complete. This is due, in all probability, to the somewhat more extensive proliferation in these specimens at the beginning of the experiment.

except that in one group iodin was given and a fish taken every second day, while in the other group, all the fish were traumatized by the inoculation and a specimen was taken every third day.

TABLE IV. *Effect of Administration of Iodin.*

No. of fish.	Date specimen taken.	Age in years.	Histological condition of thyroid.			Classification.
			Stainable colloid.	Distribution, infiltration, etc.	Epithelium.	
1 Male	Oct. 11	1⅔	Present throughout—reduced	Throughout all the structures beneath the pharyngeal mucosa including bone and muscle	Columnar	Marked glandular hyperplasia
2	Oct. 13	1⅔				
3 Female	Oct. 15	1⅔	Present throughout—reduced	Throughout all the structures beneath the pharyngeal mucosa including bone and muscle	Columnar	Marked glandular hyperplasia
4 Female	Oct. 17	1⅔	Present throughout—reduced	Throughout all the structures beneath the pharyngeal mucosa including bone and muscle	High cubical	Colloid—moderate marked glandular hyperplasia, involuting (reverting)
5 Male	Oct. 19	1⅔	Present throughout—slightly reduced	Throughout all the structures beneath the pharyngeal mucosa including bone and muscle	High cubical	Colloid—moderate glandular hyperplasia, involuting (reverting)
6 Male	Oct. 21	1⅔	Present in all follicles—slightly reduced	Throughout all the structures beneath the pharyngeal mucosa including bone and muscle	Low columnar	Colloid—moderate glandular hyperplasia, involuting (reverting)
7 Female	Oct. 23	1⅔	Present in all follicles—nearly normal	Throughout all the structures beneath the pharyngeal mucosa including bone and muscle	Cubical	Colloid—early moderate glandular hyperplasia, involuting (reverting)
8 Female	Oct. 25	1⅔	Normal throughout	Throughout all the tissues beneath the pharyngeal mucosa including bone, muscle and extensions into gill segments	Low cubical	Colloid—early glandular hyperplasia, involuting (reverting)

TABLE IV. *Effect of Administration of Iodin.* (Continued).

No. of fish.	Date specimen taken.	Age in years.	Histological condition of thyroid.			Classification.
			Stainable colloid.	Distribution, infiltration, etc.	Epithelium.	
9 Female	Oct. 27	1⅔	Normal throughout	Throughout all the tissues beneath pharyngeal mucosa including bone, muscle and cartilage erosions	Low cubical	Colloid—early glandular hyperplasia, involuting (reverting)
10 Male	Oct. 29	1⅔	Normal throughout	Throughout all the tissues beneath pharyngeal mucosa including bone, muscle and cartilage erosions	Low cubical	Pure colloid goitre. Complete involutin (reversion)
11 Male	Oct. 31	1⅔	Present in all follicles—perhaps slightly less than normal	Throughout all the tissues beneath pharyngeal mucosa including bone, muscle and cartilage erosions	Cubical	Colloid—early glandular hyperplasia, involuting (reverting)
12 Female	Oct. 31	1⅔	Abundant in all follicles	Throughout all the tissues beneath pharyngeal mucosa including bone, muscle and cartilage erosions	Flat cubical	Pure colloid goitre, complete involution (reversion)
13 Female	Oct. 31	1⅔	Abundant in all follicles	Throughout all the tissues beneath pharyngeal mucosa including bone, muscle and cartilage erosions	Low cubical	Colloid—early glandular hyperplasia with well marked fibrous stroma, involuting (reverting)
14 Female	Oct. 31	1⅔	Abundant in all follicles	Throughout all the tissues beneath pharyngeal mucosa including bone, muscle and cartilage erosions	Low cubical	Pure colloid goitre, complete involution (reversion)
15 Male	Oct. 31	1⅔	Abundant in all follicles	Throughout all the tissues beneath pharyngeal mucosa including bone, muscle and extensions into the gill segments	Low cubical	Pure colloid goitre, complete involution (reversion)

When one compares the rapidity of the reversion process in mammals with fish, it is seen that mammalian hyperplasias react much more quickly with iodin. The question arises, would fish glands react more quickly if iodin could be given direct (by mouth or subcutaneously) as is done with dogs. Perhaps to a slight extent the process could be hastened, but it seems more likely that in these cold-blooded animals the tissue changes are normally slower. This same iodin effect was obtained with the less extensive hyperplastic (goitrous)thyroids of the Lake Erie pike.[8]

INOCULATION EXPERIMENTS.

On October 10, fifteen one and two-thirds years old fish from House XI, Tank 36, were inoculated.[9] Seven were inoculated with a tumor from a one and two-thirds years old fish, and the remaining eight with a tumor from a two and two-thirds years old fish. Histologically both tumors were identical. They were typical examples of hyperplasia with very scanty colloid substance, marked infoldings and plications and the epithelial tissue had invaded the bone and muscle of the subpharyngeal region. There were also small tumor bulgings beneath the epithelium of the floor of the pharynx. Both fish had secondary (?) thyroid growths at the tips of their lower jaws.

The first specimen was taken October 13 and the last, November 24. Specimens taken October 13 and October 16 show clumps of thyroid epithelial cells at the base of the trocar wound. In none of the specimens taken later could we make out any cells comparable to thyroid cells. There was no evidence of local infection in any of the specimens. The specimens taken from October 19 to November 24 show at the inoculation site only the evidence of previous slight hemorrhage with scar formation. The thyroids of all fish used were macroscopically not enlarged, but histologically

[8] Marine and Lenhart, *Johns Hopkins Hosp. Bull.*, 1910, xxi, 95.

[9] We used the Bashford and Murray method of inoculating small cylinders of the tumor in a trocar and shoved out by means of a stylette. The inoculations were made subcutaneously just behind the shoulder girdle on the left side. The small skin wound was bathed in saturated solution of sodium chloride to prevent fungus growth at the site. Specimens were taken every day. The inoculation area and the thyroid were kept for histological examinations.

there was extensive proliferation and invasion of the bone and muscle in all. The experiment is not tabulated for the reason that the results were entirely negative. In spite of our negative results, we are rather inclined to believe that the tissue can be transplanted after we have become more familiar with the physiological requirements. The site we chose for inoculation was purely for convenience. We have never observed secondary (?) growths except in the gills and at the tip of the lower jaw, and it would seem that these locations would be more favorable for the growth of transplants. However, there is still doubt whether these growths in the gills and at the tip of the lower lip are actual metastases, or are merely the hyperplasia of existing anlagen.

ANATOMICAL DESCRIPTIONS OF THE THYROID CHANGES.

As a result of moderately extensive histological examinations of the thyroids of sea trout, sea bass, lake trout, brook trout, pike, bass, herring, white fish, carp fish, and gold fish, we have come to the following conclusions regarding the normal thyroid in these bony fish. (*a*) *The normal thyroid* (Fig. 2) in these several varieties of fish, representing widely different areas throughout the United States, shows only minor individual differences. It is also strikingly like the normal thyroid of mammals as regards the histological appearance of the thyroid unit—the alveolus or follicle. These alveoli are loosely distributed, separate or in small collections in the areolar tissues about the ventral aorta, between the first and third gill segments. Scattered follicles extend outward for slight distances on the second and third gill arches. *The gland is not encapsulated* as in reptiles, amphibia and all higher animals. This fact is of the greatest importance in the interpretation of the pathological conditions, inasmuch as isolated follicles are often seen lodged in bony angles or in the fascia of striped muscle quite distant from the main thyroid area. The main mass of thyroid tissue lies in the second gill segment and scattered alveoli in the areolar tissue extend antero-posteriorly and laterally from this central mass.

Histologically the gland is very vascular. Each alveolus is surrounded as in the mammalian gland with a rich capillary network. The alveoli are rounded and show the same variations in size as is

seen in higher animals. While the size of the alveoli, of course, varies with the size and age of the fish, as in other animals, the majority of alveoli would be included in measurements of 0.1 to 0.5 millimeter in diameter. The lining epithelium is low cubical, regularly arranged in a single layer with small darkly staining nuclei.

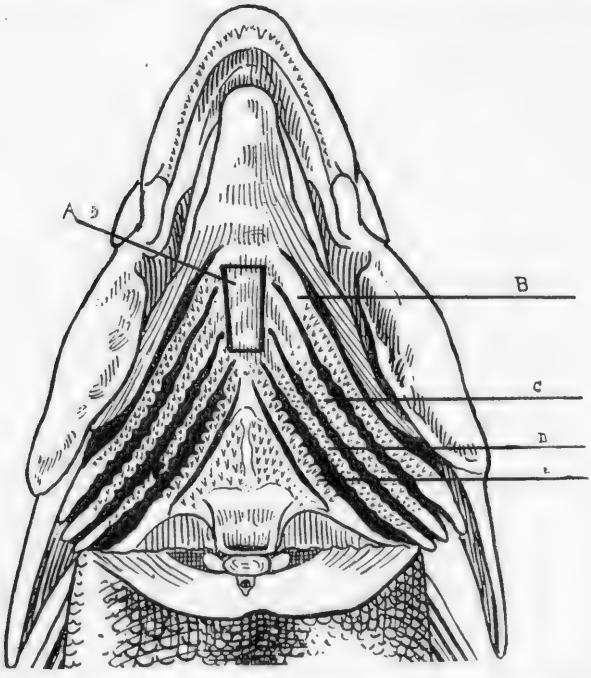


FIG. 2. Showing the location of the thyroid area in the bony fish (Teleostei). *A*, thyroid area; *B*, first gill segment; *C*, second gill segment; *D*, third gill segment; *E*, fourth gill segment.

The colloid completely fills the alveolus, stains uniformly with eosin and comes in direct contact with the lining epithelium (Plate XXV, Figs. 2 and 3). In its general histological appearance, therefore, the normal thyroid of these bony fish is similar to that of mammals in all its essential characteristics except that there is no capsule.

(*b*) *Hyperplasias:* (Plate XXV, Fig. 3 and Plate XXVI, Fig. 4). In the development of any true goitre the thyroid undergoes certain characteristic changes which are designated by the term "hyperplasia." These changes in the fish thyroid are similar in all their essential features to the hyperplasias in mammals as typified by the

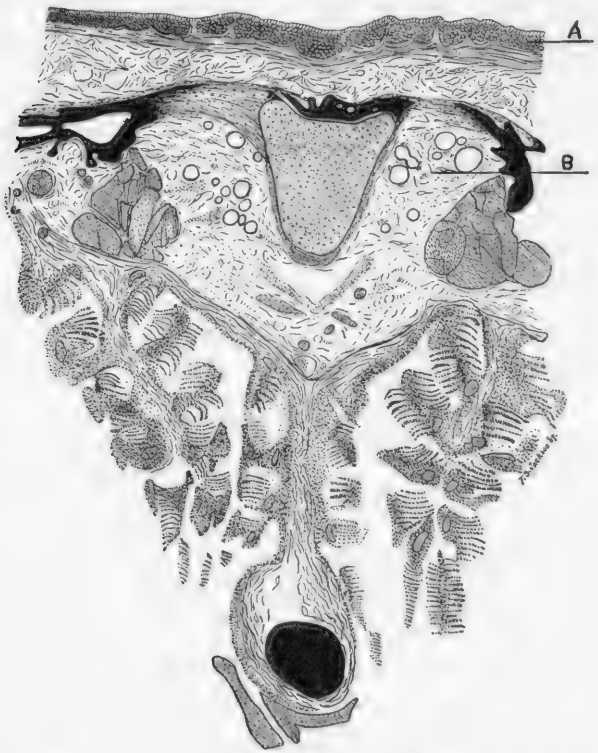


FIG. 3. The distribution of the normal thyroid follicles in the brook trout as seen in a transverse section of the thyroid area at the level of the second gill segment. *A*, pharyngeal mucosa; *B*, thyroid follicles.

spontaneous or induced (following partial removal) hyperplasias of dogs or the hyperplasia of exophthalmic goitre in man. Age is the same important factor in fish as in mammalian hyperplasias, in that the *young* are most severely affected. As in the higher animals

so with fish, there are all degrees of active thyroid hyperplasia ranging from the slightest departure from normal (hypertrophy) to the marked glandular hyperplasias. As will be reported elsewhere, this active hyperplasia of fish thyroids in a mild form occurs in certain Lake Erie fish in their wild state, but the severer grades going on to visible tumor formation have been thus far observed particularly in the carnivorous fish (trout, salmon) reared in captivity, although it may occur in any place where proper conditions exist.

The most striking histological changes are the same as in mammalian thyroids and are seen in the *blood vessels,* the *stainable colloid* and the *epithelium.* The capillaries and larger vessels of the stroma are dilated and hypertrophied. The stainable colloid is decreased but slightly in the early stages of hypertrophy, while it may be entirely absent in the marked degree of hyperplasia. The epithelium lining the alveoli varies from cubical in the mild degrees of hypertrophy to high columnar in the marked degrees of hyperplasia. The infoldings and plications of the lining epithelium are present in all the more marked degrees, but, perhaps, to a less extent than in similar degrees of hyperplasia in mammals. This may, perhaps, be explained by the absence of the restraint normally exerted by the gland capsule of higher animals. It is thus seen that the hyperplasias of fish are similar in all their essential characteristics (anatomical structure, mode of development, etc.) to those seen in mammals, just as the normal fish thyroid is similar to the normal mammalian thyroid. There is, however, one important minor difference that has figured prominently in the histological interpretation and significance of the active thyroid hyperplasia of certain fish, namely, the brook trout and salmon. This difference is that the fish thyroid is not encapsulated while the thyroids of all animals from amphibia up are enclosed in a definite fibrous membrane. In mammalian goitre, it has been established that ordinary thyroid hyperplasia does not penetrate this capsule, but that the capsule and stroma hypertrophy are parallel with the epithelial proliferations, so that if the thyroid growth penetrates the capsule and invades surrounding structures, such manifestations are taken as positive signs of malignancy. This same standard cannot be applied to fish thyroids where normally the gland is without any capsule and the alveoli widely dis-

tributed in the areolar space around the ventral aorta. By studying a large series, it is easily demonstrated that this areolar space is always filled before there is evidence of thyroid growth into bone, muscle, etc. It is also clear that when a gland whose normal follicles are so widely scattered (involving bone and muscle, etc.) undergoes hyperplasia the histological picture would present the appearance of actual invasion of the structures adjacent to which the normal follicles were located. This apparently wild, aimless growth is present as well in the early stages of hyperplasia as in the late stages. The differences are only in the degree and extent of the atrophy and absorption of these contiguous structures as bone, muscle and cartilage. Out of some four hundred and fifty specimens examined histologically *from the tanks,* representing ages from eight months to two and two-thirds years, the epithelial growth had involved all the structures beneath the pharyngeal mucosa in *every* specimen. On the other hand, there was not a single instance where the growth had penetrated the skin by actual invasion. The skin over a growing thyroid tumor is thinned by pressure atrophy and gradually gives way to ulceration when the actual break in continuity occurs.

Pick has emphasized the fact that there are several histological types of the tumor growth which he has likened to the variations seen in the inoculable breast tumor of mice. One of these types is associated with a great increase in connective tissue and gives the appearance of a scirrhus carcinoma in which the epithelial elements grow in columns and strands and are not differentiated into actual alveoli. This scirrhus effect is never seen in the early stages of the proliferation, but becomes evident when the growth has extended to the more resistant structures as bone and skin. In all the cases where the epithelial proliferation has extended to the skin, muscle or bone, there is a zone of fibrous overgrowth with the consequent destruction and distortion of the epithelial elements. Aside from this localized and peripheral sclerosis there is also in older fish a less marked general increase in the stroma that becomes evident in the cases undergoing or which have undergone spontaneous involution (reversion) to the colloid state. There are other types which represent all the stages of the (reversion) involution from the marked

hyperplasias with no colloid and high columnar epithelium down to quite uniform colloid transformations with cubical epithelium and abundant colloid. Pick has roughly divided these stages of thyroid change into two types which he designates as *hetero-* and *homo-* types of carcinoma. By "heterotype" he means those forms in which the evidence of active glandular hyperplasia with reduced colloid and high columnar epithelium predominates, and under "homotype" he includes all forms partaking of the colloid or normal type of gland in which there are well marked accumulations of colloid and the epithelium is cubical.

To one familiar with the cycle of changes that the thyroid follicles undergo in the developing hyperplasia and its involution (reversion) to colloid goitre during the stage of recovery, it is not necessary to introduce these terms to explain the several histological stages of the process. The observed histological phenomena are more rationally explained by comparing them with the similar phenomena of hyperplasia and involution as seen and studied in the mammalian thyroid.[10]

(*c*) *Colloid glands* (Plate XXVI, Figs. 5 and 6) in fish do not differ in any essential way from similar glands in the higher animals, nor do they differ from normal glands in any essential way except in size and in showing the vestiges of previous active hyperplasia. These marks of a previous hyperplasia, however, are well defined and characteristic. The changes are to be found in the size, shape and distribution of the follicles in the blood vessels and stroma. The distribution of the follicles is that attained during the stage of active hyperplasia and in examples that have been preceded by marked hyperplasia, the rounded follicles are present in bone, muscle and all the tissues beneath the pharyngeal mucosa, including the extensions out along the gill arch arteries into the gills. The follicles are everywhere filled with colloid which is normal in appearance and distribution. The epithelium lining the follicles is normal (flat cubical). The stroma is increased, although to this statement there are exceptions depending on the extent of the fibrosis occurring during the period of active hyperplasia and the extent of the absorption which normally occurs during the process

[10] Marine and Lenhart, *Bull. of the Johns Hopkins Hosp.*, 1909, xx, 131.

of involution (reversion). The blood supply which is so markedly increased in the active hyperplasia is reduced to conform with that of a normal gland. The process is similar to that obtaining in mammals, namely, a physiological endarterial thickening. The histological appearance of colloid glands in fish is, therefore, similar in all respects to colloid glands occurring in mammals, whether of spontaneous origin or induced by the action of iodin. The important question is, whether there is a stage in these hyperplasias that will not react with iodin or undergo spontaneous recovery when removed from their unfavorable environment. Experiments are now being made to ascertain, if possible, to what degree the hyperplasia can go and still remain susceptible to physiological reactions. If a stage in these hyperplasias can be demonstrated when they do not react to iodin or to favorable changes in environment, then a diagnosis of carcinoma would seem justifiable. It would seem possible that such a stage may be found and, as pointed out above, would be in harmony with our present views of cancer development. It seems to be definitely established that true cancer of the thyroid in dogs and man does not show the characteristic reaction with iodin or to environmental changes.

(*d*) *Circumscribed Tumor Formations* (Plate XXVI, Figs. 7 and 8).

Pick[11] has pointed out the generally symmetrical development of these fish goitres, just as is true of all true goitres in all animals. There may develop, however, in all mammalian goitres rounded or circumscribed masses apart from the main body of the goitre. These are usually called simple adenoma or foetal adenoma, depending on the histological appearance. Now and then one sees in these fish goitres similar rounded and symmetrical circumscribed growths which histologically may be as fully differentiated as the surrounding ordinary hyperplasia, but more commonly they are less well differentiated, as evidenced by the lack of regular uniform types of cell, or the more marked infolding and piling up of epithelial elements and lessened stroma—all these factors being interpreted as evidence of a more rapid growth and a greater physiological independence than the ordinary surrounding hyperplasia. Whether

[11] Pick, *loc. cit.*

these more independent growths in fish goitres react with iodin or not has not been ascertained, as it was not our fortune to have included any such specimens in the iodin experiment. As is well known in mammals, these growths do not react either with iodin or spontaneously in any constant manner, although they do in most cases return to the resting state after variable periods of growth, while occasionally they continue their growth with manifestations of carcinoma. The circumstances that determine continuation or cessation of growth are unknown to us and are quite beyond any known method of physiological control. This is in sharp contrast with the simple goitrous hyperplasias which are under definite physiological control.

CLINICAL MANIFESTATIONS OF GOITRE IN FISH.

We could make out no conditions comparable to the cancer cachexia seen in higher animals. The fish on the contrary are abnormally large for a given age, excessively fat, and in contrast with their fellows living wild in the streams, are *weak, sluggish, lumbersome* creatures with little resistance when taken by hook and line. They also die more quickly than normal fish following their removal from the water. Plehn[12] thought she could make out evidences of cancer cachexia. Death, we believe, rarely results directly from the disease but through intercurrent infectious processes, particularly gill infections, fungus, etc. The major direct effect of the tumor seems to be mechanical. Thus the locking of the gills prevents or lessens normal respiratory movements and ability to feed. As to the duration of life in these affected fish, we have no complete observations. Smith[13] has lately recorded his observations as to the effect of goitre in fish and our observations above recorded are in harmony with them. He also adds that the affection does not seem to shorten the life of the fish to any appreciable extent. In general, this may be true of the thyroid effect *per se,* but the affected fish are certainly more susceptible to intercurrent disease and it indirectly shortens life.

Finally, some mention should be made of associated changes in

[12] Plehn, *loc. cit.*

[13] *Washington Med. Annals,* 1909, viii, 313.

these goitrous fish. We have already mentioned the fact that these fish are large for their age, excessively fat and, as a rule, show excessive skin pigment, and even in the thyroid and internal organs there may be pigment development. There is also constant *splenic* and *lymphoid enlargement* just as is so often seen in mammalian goitre. The question of the blood condition has not been investigated.

SUMMARY AND DISCUSSION.

Is this thyroid hyperplasia true carcinoma? In view of the facts that young fish are more affected than old fish, that iodin stops the hyperplasia and causes it to return to the colloid state, that removal of the fish from the ponds to the open brook also affects a cure, that the clinical incidence of tumors is directly related to the water supply, and that the severity of the disease as determined by histological examination is likewise directly related to the water supply, we cannot accept the prevailing opinion that the disease is true cancer. On the other hand, we believe that the disease as studied by us is an extreme illustration of endemic goitre, the end stage of which is cretinism. The opinion that it is cancer is based purely on anatomical studies—the essential fact being that the growing thyroid invades bone, muscle, etc. Such findings in mammalian tissues are strong evidence of malignancy, but this standard cannot be applied to fish thyroids for the reason that any growth of a widely and loosely distributed nonencapsulated gland would give the histological appearance of invasion of the surrounding tissues.

The general views as to the cause of the disease may be divided into two groups: (1) infectious and (2) metabolic.

Taking up the infectious theory, there are abundant grounds for such a view when one considers the flora of the tank. The very careful histological examinations by Hofer, Bonnet and Pick have yielded uniformly negative results. Direct infection cannot be excluded, but the fact that fish placed in the tail-race recover spontaneously, although living in the theoretically most polluted water, is not in harmony with our present conception of water-borne infection.

Taking up the metabolic theory, the three factors of overfeeding, overcrowding and limited proportional water supply together with the many possibilities into which they are divisible seem to us to be in some way, still obscure, directly concerned with the development of the disease. It is not probable that a single substance, whether the result of food decomposition or of excretory products, excites the thyroid to its activity but rather that the thyroid reaction is the result of the activity of a great variety of these products.

METHODS OF CURE AND PREVENTION.

Although the essential cause of thyroid hyperplasia is still unknown, there are two well established methods applicable to fish as well as mammals.

The first is the hygienic or natural method and consists of reproducing natural environmental conditions as far as possible. In particular, *overfeeding* and *overcrowding* should be avoided and the *water supply* should be ample to take away all the excreta and the decomposing food.

The second method consists of the use of iodin-containing substances.

CONCLUSIONS.

1. The so-called carcinoma of the thyroid of brook trout is, in its early stages at least, nothing more than severe endemic goitre. The possibility that this physiological hyperplasia may, in some instances, go over into actual carcinoma is not denied and is in harmony with the more modern views of cancer development.

2. *Overfeeding* and *overcrowding* and a *limited water supply* are the three major factors in the production of filthy, unhygienic tanks or ponds and these unsanitary, unhygienic and filthy tanks are in a very important but still unknown manner associated with the development of thyroid hyperplasia.

3. Prevention and cure is favored by adjusting the amount of food and the number of fish to the water supply or by the addition of iodin containing substances to the water passing through the ponds.

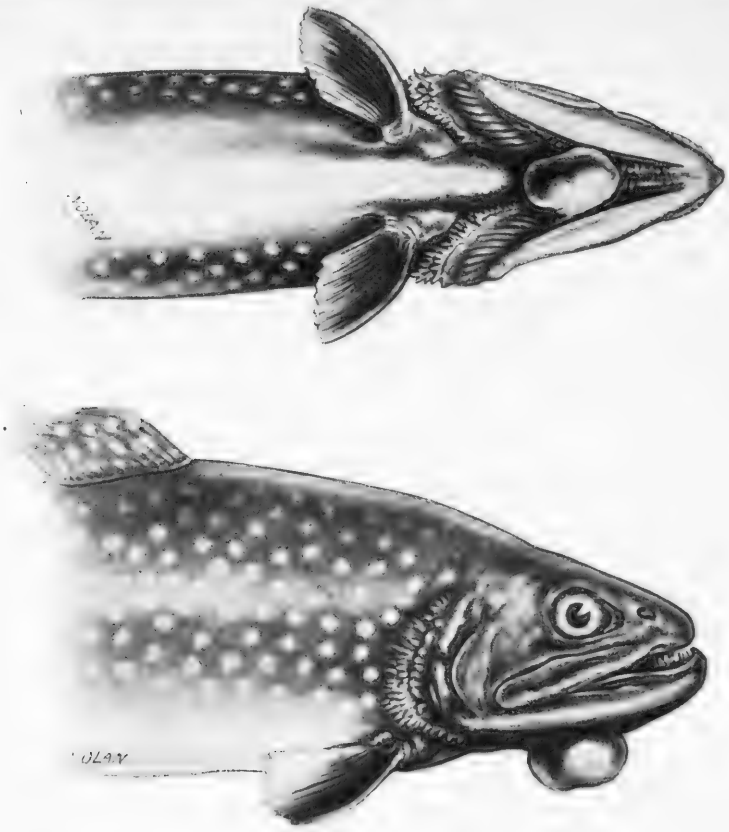
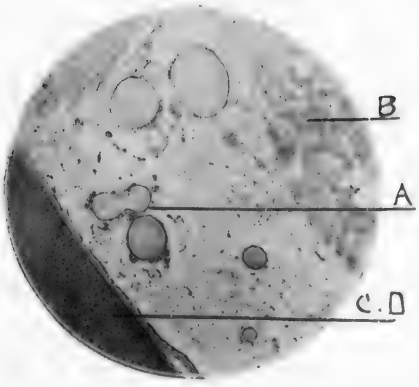


FIG. 1.



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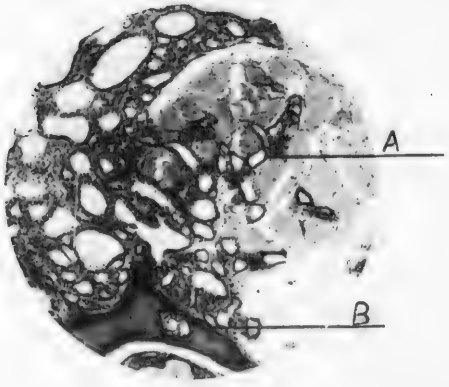

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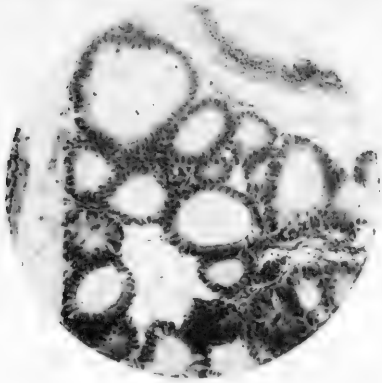

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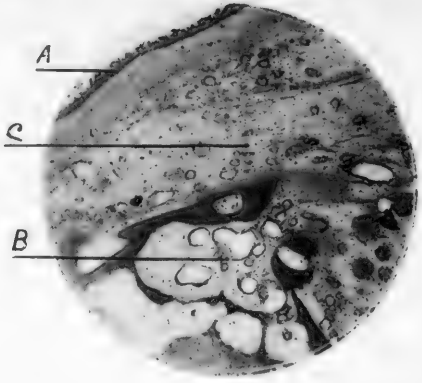

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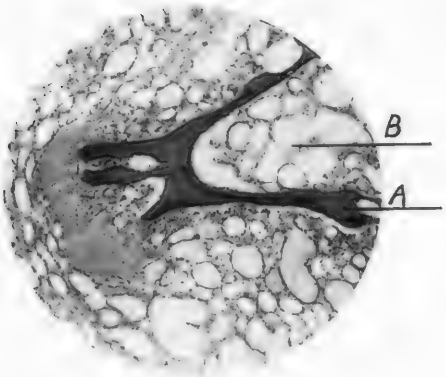

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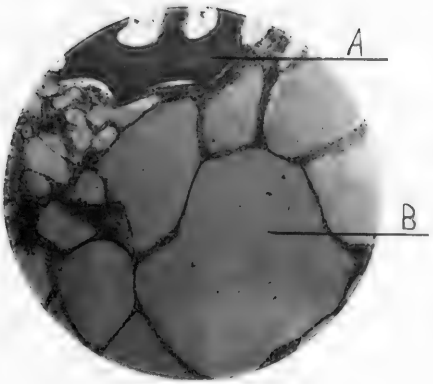

FIG. 7.

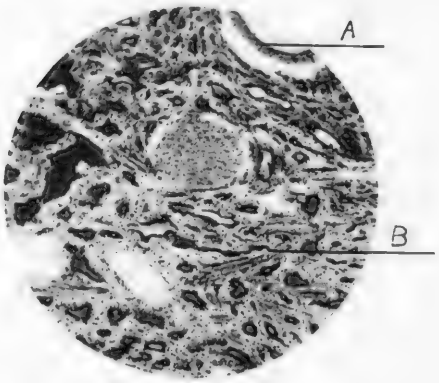

FIG. 8.

This study was made possible through facilities granted by and through the coöperation of the Honorable W. E. Meehan, Commissioner of Fisheries for the State of Pennsylvania. We wish to express our obligations and thanks to Mr. Meehan and also to the officers of the private club at whose hatchery our experiments and observations were made.

EXPLANATION OF PLATES.

Plate XXV.

Fig. 1. Showing the location, size and relations of an external visible goitre of the brook trout. Note also the swollen and hypertrophied condition of the gills—so-called "soregill," and the great increase in skin pigment and the absence of the red and yellow color spots.

Fig. 2. Normal trout thyroid showing the absence of a capsule, the scattered arrangement of the follicles and their relation to striped muscle, bone and cartilage. *A*, thyroid follicles; *B*, striped muscle; *C*, bone; *D*, cartilage.

Fig. 3. Active thyroid hyperplasia showing *A*, invasion of striped muscle; *B*, relation to bone.

Plate XXVI.

Fig. 4. Active hyperplasia showing the regular uniform arrangement and the high columnar type of the follicular epithelium and absence of colloid.

Fig. 5. Colloid goitre from a three and two-thirds years old fish from the trout stream showing spontaneous recovery, notwithstanding the invasion of bone and subpharyngeal tissues. *A*, pharyngeal mucosa; *B*, typical colloid follicles in bony angles; *C*, typical colloid follicles in the subpharyngeal tissues.

Fig. 6. Showing the effect of iodin on the marked active hyperplasias. Note that all follicles are lined with flat cubical epithelium, that all follicles contain colloid, and that the thyroid tissue has invaded all the structures beneath the pharyngeal mucosa. *A*, bone; *B*, thyroid follicles in bone that have completely reverted to the colloid or quiescent state.

Fig. 7. A large external goitre illustrating the relation of follicles to bone. *A*, bone; *B*, large colloid follicles of the reverted thyroid hyperplasia.

Fig. 8. A large external goitre illustrating the atrophy and degeneration of the follicular epithelium with the extreme fibrosis giving the appearance of scirrhus carcinoma. This is exactly similar to the cretinoid or myxodematous atrophy in mammals. *A*, artery; *B*, distorted follicles in the fibrous stroma overgrowth.

THE COMPLEMENT BINDING TEST IN RABIES.[1]

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The work of A. Wasserman and his collaborators in the application of the complement binding test of Bordet and Gengou to the diagnosis of syphilis and other diseases has been followed, as is well known, by a long series of investigations, and many observers have confirmed the views of Wasserman. Positive reactions are said to have been found in typhoid, tuberculosis, meningitis and many other diseased conditions. The most striking results have been obtained in the study of syphilis.

Many views have been expressed with regard to the nature of the reaction. The original claim that specific antigens as well as antibodies could be demonstrated in this manner was soon abandoned, since it was found that the test was obtained not only with specific, but also with normal extracts. The fact that alcoholic extracts were found to be effective showed the active substance to be alcohol soluble or lipoid in nature. Porges and Meier, working under Wasserman's direction, found that pure lecithin was able to bind complement with syphilitic sera, and it was evident later that a large number of other lipoids acted as well or even better than lecithin. Meantime Landsteiner and Stankovic had obtained similar results with numerous organically suspended and colloid dissolved substances: Axamit, working with bacterial extracts, had found that these alone bind complement, while Uhlenhuth had found binding with a long list of the most unrelated and diverse materials.

None of these results have been held to affect the value of the test in the opinion of its supporters, since it is their claim that complement binding can be obtained only by the action of specific, never of normal sera, or, if the contrary is ever true, it is said to be

[1] Received for publication February 8, 1910.

so rare that it can be practically disregarded. These views have not met with universal acceptance, however, since opposite results have been found by several prominent investigators. Ranzi obtained binding of complement with normal sera alone, with extracts of tumors alone, and with tumor extracts combined with normal sera. Weil and Braun found binding to the same extent with normal as with specific sera together with tumor extracts. They considered the active agent to be not specific in nature, regarding it as probably a substance derived from degenerative cell changes in various diseased conditions. Much, testing sera from twenty-five cases of scarlet fever with syphilitic liver extract found binding in ten. He obtained a similar reaction in many other diseased conditions and also with normal sera, and together with Eichelberg he regarded the test as without diagnostic value.

In 1907, Heller and Tomarkin (1) sought to demonstrate the existence of specific antibodies against rabies virus in the serum of animals immunized against rabies. They used the sera of rabbits inoculated subcutaneously on twenty-four consecutive days with extracts of fixed virus rabbit cords made from two to eight days after death, testing sera two to three weeks after the last injection. As test fluid, expressed juice of rabbit's brains obtained under pressure of 350 atmospheres was used, the animals having died from fixed or street virus which were tested separately. All customary controls were used, and the experiments were frequently repeated with varying amounts, but results were negative as to diagnostic value of test in rabies. Occasionally, increasing inhibition of hemolysis was obtained by the use of increasing amounts of specific immune juice and immune sera, but the same result was found upon using immune sera with expressed juice of normal brains, or normal sera together with specific or normal expressed juices.

Friedberger (2), from a theoretical standpoint, thought this test especially well adapted for rabies. He used the serum of a horse inoculated with extract from a fixed virus rabbit brain, the serum of a rabbit immunized with horse blood, guinea pig complement, and customary controls. He found a small amount of binding with rabies serum, but no stronger than with serum of normal horse and

came to the same conclusion as Heller and Tomarkin, that no positive conclusions could be based upon this test in rabies.

A third report of complement binding tests with rabies was published after the completion of the present experiments. Baroni (3) and his associates used three sera from animals immunized to rabies. Two were from rabbits inoculated with fixed virus rabbit cord emulsion, the first rabbit having died after six subcutaneous inoculations, the last one being made into the anterior chamber of the eye; the second rabbit resisted a similar eye inoculation, after a previous intravenous injection. The third serum, from Marie, was from a sheep given multiple injections of fixed virus at the Pasteur Institute. The three sera were tested against spleen and brain of rabbits immunized as above, with fresh guinea pig complement, according to the classical method, but, notwithstanding numerous trials, no binding of complement could be demonstrated, hemolysis being always identical with that in controls of normal rabbit serum.

At the suggestion of Dr. Williams, the writer undertook the present series of experiments with rabies.

The rabies material tested consisted of extracts of the brains of fixed virus rabbits, the rabbits being those which furnished the cords used in the treatment of rabies patients. The fresh brain material was crushed in a sterile tube, mixed in proportion of 1:4 with normal salt solution containing 5 per cent. of carbolic acid, agitated at room temperature for twenty-four hours, and centrifuged for from one to two hours, when the supernatant fluid was drawn off into sterile flasks and placed in the ice-box until needed. Extracts were made in the same manner from the brains of normal rabbits.

A series of normal rabbits were inoculated subcutaneously with rabies material. Rabbit A, for the first few inoculations, was injected with the above fixed virus extracts, and later, this rabbit and all others of the series were injected at intervals of ten days with an emulsion of fresh fixed virus brain in salt solution, beginning with 0.25 cubic centimeter and increasing 0.2 with each treatment up to the amount of 2.5 cubic centimeters at the last inoculation. After the fourth treatment the serum of these rabbits was used with the fixed virus brain extract for the complement binding test. Control tests were made with the serum of normal rabbits, and both rabies and normal sera were tested against the normal brain extract by the same method.

The blood cells used were those of the horse washed four times with salt solution, a series of rabbits having been immunized against horse blood by subcutaneous inoculations of 10 cubic centimeters of washed horse blood cells at ten day intervals. One of these rabbits developed sufficient hemolysin after the third inoculation to dissolve 1 cubic centimeter of a 5 per cent. suspension of horse blood cells with 1/1000 cubic centimeter of serum. This amount gradu-

ally decreased until 1/200 cubic centimeter of serum was required for the same amount of blood at the time of the last experiment. The amount of hemolytic serum used in each test was twice that needed for blood solution, the quantity having been ascertained by preliminary test. All sera were inactivated by heating at 56° C. for one half hour. The complement used was furnished by fresh, normal guinea pig serum. The various substances were first diluted with normal salt solution to such strength that 1 cubic centimeter of each could be taken for the test, making 5 cubic centimeters in all. When one or more substances were omitted, tubes were filled to the same level with normal salt solution.

Thus diluted, the fixed virus or normal extract and fixed virus or normal serum were measured into sterile tubes, the complement added and the tubes placed in the incubator for two hours, and then in the ice-box. The next morning the tubes were thoroughly shaken and again placed in the ice-box until the following morning, when the final reading was made.

The experiments were many times repeated, using sera from four fixed virus rabbits, and from two normal rabbits, together with extracts from four fixed virus rabbits and from four normal rabbits, in varying amounts and combinations, together with controls as shown in table. In this way over fifty tests were made, showing in all the same result—complete or practically complete inhibition of hemolysis. No difference whatever could be perceived between the action of normal and of fixed virus serum, except that in one series a slightly stronger binding power was shown by the normal than by the fixed virus serum. In one case the normal serum and normal brain extract were both obtained from the same rabbit, but the result here was identical with that in all the other tests. The slight hemolytic action of normal guinea pig serum upon horse blood corpuscles, frequently noted, was not sufficiently marked to interfere with the definite comparison of results, the same being true of the slight inhibitory action sometimes seen in both fixed virus and normal extracts. Inhibition was always complete as to the action of the hemolytic serum, with invariable full hemolysis in hemolytic system controls.

The following table is representative of results obtained.

These experiments agree with those of former investigators, in showing the absence of any specificity for rabies in the complement binding test.

That the normal sera should have shown an equal inhibitory power to that of the fixed virus sera remains unexplained, but it is

TABLE I.

Sera. Fixed virus rabbit. Normal rabbit.		Extracts. Fixed virus rabbit brain. Normal rabbit brain.			Result.
Serum.	Extract.	Complement 1:10.	Hemolysis amboceptor 1:100.	Blood 5 per cent.	
F. V. rabbit .2 c.c.	F. V. extract .2 c.c.	1 c.c.	1 c.c.	1 c.c.	Practically complete inhibition
" " .1 "	" " "	"	"	"	Nearly complete inhibition
" " .01 "	" " "	"	"	"	Complete hemolysis
" " .006 "	" " "	"	"	"	" "
" " .005 "	" " "	"	"	"	" "
" " .2 "	Norm. extract .2 c.c.	"	"	"	Practically complete inhibition
" " .1 "	" " "	"	"	"	Nearly complete inhibition
" " .01 "	" " "	"	"	"	Complete hemolysis
" " .006 "	" " "	"	"	"	" "
" " .005 "	" " "	"	"	"	" "
Norm. rabbit .2 "	F. V. extract .2 c.c.	"	"	"	Practically complete inhibition
" " .1 "	" " "	"	"	"	" " "
" " .01 "	" " "	"	"	"	Complete hemolysis
" " .006 "	" " "	"	"	"	" "
" " .005 "	" " "	"	"	"	" "
" " .2 "	Norm. extract .2 c.c.	"	"	"	Practically complete inhibition
" " .1 "	" " "	"	"	"	" " "
" " .01 "	" " "	"	"	"	Complete hemolysis
" " .006 "	" " "	"	"	"	" "
" " .005 "	" " "	"	"	"	" "
F. V. rabbit .2 "	—	"	"	"	" "
" " .4 "	—	"	"	"	" "
Norm. rabbit .2 "	—	"	"	"	" "
" " .4 "	—	"	"	"	" "
—	F. V. extract .2 "	"	"	"	Complete hemolysis; occasional slight inhibition
—	" " .4 "	"	"	"	Complete hemolysis; occasional slight inhibition
—	Norm. extract .2 "	"	"	"	Complete hemolysis; occasional slight inhibition
—	" " .4 "	"	"	"	Complete hemolysis; occasional slight inhibition
F. V. rabbit .4 "	—	—	—	"	No hemolysis
Norm. rabbit .4 "	—	—	—	"	" "
—	F. V. extract .4 "	—	—	"	" "
—	Norm. extract .4 "	—	—	"	" "
—	—	—	1 c.c.	"	" "
—	—	1 c.c.	—	"	Slight trace hemolysis
System control.		1 c.c.	1 c.c.	1 c.c.	Complete hemolysis

felt that errors of technique and merely accidental results are ruled out by the number of experiments made and by the confirmatory action of the numerous controls. The complete hemolysis obtained with all amounts of serum below 0.1 cubic centimeter would seem

to exclude precipitins here as the probable cause of complement binding.[2]

An extensive bibliography upon the complement binding test is given by Fleischmann (4). No other references are given except those relating to the test with rabies.

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[2] To the above a few additional tests may be added. Serum from a normal rabbit, 0.2 and 0.4 cubic centimeters, has been brought together with extract of normal rabbit brain (from same individual), 0.2 cubic centimeter, with hemolytic amboceptor from rabbit, with fresh guinea pig complement and with sheep corpuscles. Result:—partial hemolysis (0.8) with smaller dose of serum; slight hemolysis (0.3) with larger dose. The experiment repeated, substituting extract of brain of rabbit with rabies instead of normal extract, shows slight hemolysis (0.3) with smaller dose and slight trace of hemolysis (0.1) with larger dose. The same experiments in which sera from two normal guinea pigs have been tested together in place of rabbit serum, shows complete hemolysis. There is no trace of inhibition with either extract. These results with rabbit's serum constitute the first instance in this series of tests in which any inferior binding power has been shown by normal rabbit's serum with normal brain extract, as compared with brain extracts of rabbits with rabies. In this instance an additional factor has to be taken into account, namely, the normal serum and normal brain extract have been obtained from the same animal.

Thanks are due to Dr. Williams for advice and suggestions.

AN EXPERIMENTAL COMPARISON OF TRANSPLANTED TUMOR AND A TRANSPLANTED NORMAL TISSUE CAPABLE OF GROWTH.[1]

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Plates XXVII–XXX.

Much is now known of the behavior of transplantable tumors in the new host, of the conditions which regulate transplantation, and of the phenomena immediately concerned in growth or death of the introduced neoplasm; but there have been few attempts to determine what is here peculiar to tumor, as apart from that common to transplanted tissues in general. L. Loeb[2] has recognized the problem as important, and in a series of papers has dealt with some of the points involved: his work on the experimental production of deciduomata is of special note. More recently Schöne[3] has attempted an experimental comparison of tumor-growth and tissue-growth. But the method he employed, namely, the transfer of skin from the back of one mouse to another, proved ineffective, since the graft was seldom successful except on a blood-related individual. Bashford[4] in summing up our present knowledge of cancer points out that there exists a considerable parallel between its behavior and that of normal tissue. Yet when all is said, the new facts learnt by tumor-transplantation remain to-day in large part isolated. Their relation to the facts of tissue-transplantation has not been worked out.

For comparative experiments to this end it would seem advisable to use a normal tissue, which, when introduced into a new host, will

[1] Received for publication February 1, 1910.

[2] L. Loeb, *Proc. of the American Philos. Soc.*, 1908, xlvii, 3; *Arch. f. Entwcklngsmechn. d. Organ.*, 1907, xxiv, 638; 1909, xxvii, 73, 89.

[3] G. Schöne, *Beitr. z. klin. Chir.*, 1908–9, lxi, 1.

[4] E. F. Bashford, *Lancet*, 1909, lxxxvii, ii, 700.

not only gain a foot-hold but will grow, at least for a time. Only so can be tested the general laws regulating tissue-growth in the new environment, as well as those having to do with the success of transplantation. For such reason I have made use in the following experiments of material obtained from embryos.

That embryonic tissue when introduced into a new host will grow for a period is well known, and it has also been abundantly proven that sooner or later growth ceases and retrogression ensues. No unlimited growth such as is characteristic of true tumors has been observed; and this despite the fact that to produce such a phenomenon has been the goal of nearly all workers with embryonic material from Zahn[5] in 1879, to Petrow[6] and Neuhauser[7] of the present day. The ability to grow without limit remains peculiar to tumor; and a consideration of this special character can enter only indirectly into the comparison which follows.

For my purposes the use of embryos from animals of a species subject to transplantable tumors was obviously best, since thus it was rendered possible to conduct on one individual parallel observations with both tissues. Practically, the choice lay between rats and mice. But rat embryo when introduced into a new host will develop for as long as six months (Askanazy,[8] Fichera[9]), which largely unfits it for experimental uses. Mouse embryo, as I have found, grows for only a week or ten days when introduced into the subcutaneous tissue of another mouse, and retrogression is so rapid that within a month often no trace of it is left. Further, its growth is not great,—usually to only three or four times the size of the mass injected,—which renders the labor of serial sections comparatively slight. In any case a mass obtained by hashing together the embryos was to be employed; and, because those of the mouse are so small, the mixture of tissues present in each tiny graft (0.02–0.04 cubic centimeters of the mass) is more representative than is an equal amount of a mass made from rat-embryos at the same developmental period. For these reasons mice were decided upon.

[5] F. W. Zahn, *Arch. f. path. Anat. u. Physiol.*, 1884, cxv, 369.
[6] N. N. Petrow, *Beitr. z. path. Anat. u. allg. Path.*, 1909, xlviii, 1.
[7] H. Neuhauser, *Deutsche med. Woch.*, 1909, xxxv, 332.
[8] M. Askanazy, *Verhandl. d. deutschen path. Gesellsch.*, 1907, xi, 39.
[9] G. Fichera, *Arch. de méd. expér. et d'anat. path.*, 1909, xxi, 617.

In mouse-embryos 1 to 1.6 centimeters long the bones are not yet calcified, and the tissues in general are so soft that even when coarsely chopped they pass with ease through an injection-needle of 0.5 to 1 millimeter bore. But they were finely hashed with sterile knives, and carefully mixed. No fluid was added. The resulting mass could be handled in a small syringe with considerable quantitative accuracy. Being semi-solid it remained "placed" after introduction into the subcutaneous tissue. Embryonic material from different litters was never mingled.

When a series of adult, white mice are killed seven days after the injection into each, beneath the skin of the side, of the same quantity of such a mass, great differences between the grafts are observable. In all cases the implanted tissue is sharply defined and easily recognizable, adherent to the skin when that is stripped away from the muscle. But in some animals it has developed into a discrete, elastic nodule that consists microscopically of small, clear cysts and translucent bits of cartilage, embedded in an abundant stroma; whereas in others, growth has failed to take place, and there is found only a small "pocket" of opaque fragments in a connective-tissue sac. All gradations between these extremes may be observed.

It is natural to attribute such differences in result to differences in the constitution of the original grafts, composed, as these are, of varying mixture of embryonic tissues. But the secondary nature of this factor is demonstrated when several injections of the embryo mass have been made in each animal. All the grafts in a given individual suffer much the same fate. They may have grown well or they may not have grown at all, but in general they have varied together. The following is an instance in point:—

Seventeen white mice were injected subcutaneously on each side with 0.02 cubic centimeters of an embryo-mass. The injections were made at corresponding points, and all those on the left side were completed before those on the right side were begun. Thus the two grafts in each animal came from widely different portions of the injection-mass. After seven days the whole series was killed, and the grafts examined.

TABLE I.

Fate of Grafts A and B in Same Animal.

No.	I.		No.	II.	
	A	B		A	B
1	Good growth	Excellent growth	1	Good growth	Excellent growth
2	" "	Good growth	2	" "	Good "
3	Fair "	Fair "	3	" "	" "
4	Slight "	Slight "	4	" "	" "
5	" "	Graft dead	5	Graft dead	Slight "
6	Graft dead	" "	6	Hemorrhagic cyst	Excellent "
7	" "	" "	7	" "	Hemorrhagic cyst
8	" "	" "			
9	(Hemorrhagic)	" "			
10	Hemorrhagic cyst	Hemorrhagic cyst			

All the animals were healthy. I have found that a number of natural conditions affecting the host, such as age and nourishment, for example, act to influence the fate of the implanted material. These conditions are dealt with in detail in the latter part of this paper. Depending on them, some individuals are naturally resistant as hosts for implanted embryo and others naturally favorable.

Yet variations in the constitution of the individual grafts of hashed mouse-embryo must of course play some part in their fate, since not all of the tissues going to make up the amount injected are equally viable. The cells peculiar to the visceral organs fail to survive transplantation, and so, too, do the muscle and nerve cells. Cartilage, squamous epithelium and connective-tissue form the bulk of the successful graft,[10] and their presence or absence in the implanted mass will have much to do with its fate. But it so happens that these tissues are widely distributed in the embryo body, and hence cannot but be present throughout a well-mixed hash. In grafts that have failed to "take" and in which consequently all the tissues have died, the cartilage and squamous epithe-

[10] Other elements in the mouse-embryo which survive transplantation, though infrequently, are cylindrical epithelium, pavement epithelium (both of these forming cysts in the new host), osteoid tissue and, rarely, cubical epithelium in acini. The connective-tissue often contains fat-cells. A detailed account of the histological changes in transplanted embryonic tissue does not lie within the scope of this article. For that the extensive literature, and more especially the articles of Zahn (*loc. cit.*), Birch-Hirschfeld and Garten (*Beitr. z. path. Anat. u. z. allg. Path.*, 1899, xxvi, 132), and Petrow (*loc. cit.*) may be consulted.

lium are still easily recognizable. A study of many dead grafts has assured me that, as a rule, it is not from lack of such elements that failure takes place, nor is it because they are placed disadvantageously, at the center of the injected mass. Exceptionally one of these causes may be active; but in general the fate of the graft depends on the host.

The fact that individuals differ as hosts for transplanted embryo has been noted by other workers. Féré,[11] who experimented with chickens, and Petrow, who used guinea-pigs, refer expressly to the variation in their "receptivity." The embryonic tissue of white rats, as Askanazy demonstrated, grows well in practically every individual of this race into which it may be introduced. Here the differences in the host are expressed by differences in size of the growths. In the white mouse, as both Petrow and Schöne mention, growth of transplanted embryo is an irregular, not a constant result. They did not investigate for the cause of this. In human beings, as surgeons now recognize, the peculiarities of the individual body largely determine the success of grafts of adult tissue.

The possible relationship between "receptivity" to normal tissues and "receptivity" to tumor has not been generally recognized. Loeb, indeed, several years ago called attention to the fact that just as some tumors can be successfully transplanted only into the animal in which they originated, so too certain normal tissues will survive auto-transplantation, though not iso-transplantation. But the comparison might be successfully carried much further. Individual mice differ greatly as hosts for implanted tumor, but there is an equally great difference in their behavior as hosts for an implanted normal tissue capable of growth.

More exists here than an analogy, as becomes evident on a microscopical comparison of the grafts. It is well known that if a bit of normal adult tissue (Christiani [12]) or of tumor (Jensen,[13] Bashford[14]) is transplanted, its periphery remains living for some days, while the center of the fragment, if that be anything but minute,

[11] C. Féré, *Compt. rend. Soc. de biol.*, 1897, xlix, 988.

[12] H. Christiani, *Jour. de physiol. et de path. gén.*, 1904, vi, 476.

[13] C. O. Jensen, *Cent. f. Bakt., Orig.*, 1903, xxxiv, 28, 122.

[14] E. F. Bashford and J. A. Murray, *Second Scientific Report of the Imperial Cancer Research Fund*, 1905, p. 24.

degenerates and dies, owing to lack of nourishment from the host. Loeb in his paper on "Tumor Growth and Tissue Growth"[15] calls attention to this correspondence in events.

But the *growth* of implanted tumor depends immediately on a reaction from the host to give it a stroma and vascularize it.[16] One may ask: Is this likewise true of implanted embryo? Great stress has been laid upon the importance of the reaction in its association with tumor, because workers have found that the natural (or acquired) resistance of a host is manifested, not by any direct influence upon the tumor-cells that can be demonstrated, but by a failure to produce the stroma essential to development of the neoplasm. Does the resistance of a host to transplanted embryo involve a similar phenomenon?

A study in serial sections of many grafts removed at short intervals after their implantation enables me to answer both these questions in the affirmative. For the active growth of implanted embryo a supporting reaction on the part of the host's tissue is essential. With the appearance of such a reaction, the embryonic fragments are rapidly vascularized; the more hardy ones grow actively; an abundant stroma knits the mass; and at the end of seven days there is present a discrete nodule of living tissue, vascularized from the host (Plate XXVII, Fig. 1), and closely resembling in its general structure a successful tumor-graft of the same age (Plate XXVII, Fig. 2, Plate XXVIII, Fig. 3).

When the embryonic fragments have been introduced into an individual unfavorable to their growth, the resistance of the host is manifested in the failure to yield a supporting stroma. The fragments remain loose in the pocket of their injection, and soon degenerate and die, except for a few bits at the margin in contact with the host tissues and presumably nourished by diffusion from them. These survive for some days. The resemblance to what happens when a similar "mass" of tumor material is injected into an unfavorable individual is most striking (Plate XXVIII, Fig. 4; Plate XXIX, Fig. 5).

In more detail the fate of the embryonic material is as follows:—

[15] L. Loeb, *loc. cit.*

[16] Jensen, Bashford and Murray, *loc. cit.*

At the end of eighteen hours in the subcutaneous tissue of a new host, favorable or unfavorable, the fragments have undergone but little degeneration, though as yet there has occurred no union of them with the enclosing wall, from which they are separated by a thin layer of fibrin. In this wall a few leukocytes are observable, and some fluid has exuded into the pocket that contains the fragments. Between the twenty-fourth and the forty-eighth hour, if the host be favorable, the fragments next to its tissues have united with them by a mingling of cells, and some are already vascularized. These bits grow rapidly, and the ones that have lain free are soon embedded in new-formed tissue, and themselves have opportunity for vascularization. Quite characteristic of this vascularization is the formation of many thin-walled sinuses, greatly distended with blood. Not infrequently a sinus ruptures, with the result that the graft is replaced by a hemorrhagic cyst in which float the necrotic embryonic fragments. In some hosts the occurrence of such accidents seems to be the expression of a general tissue peculiarity. The table on "Fate of Grafts A and B in the Same Animal" affords illustration of this.

Cell-division in the growing mass is almost wholly by amitosis. Within four days the fragments are knit together by a stroma and well vascularized. A large part of this stroma is derived from the proliferated embryonic connective-tissue, which has so far assumed the appearance of that elicited from the host as to be no longer distinguishable except by its position. The cartilage has grown to ten or twenty times its original bulk; the squamous epithelium has formed cysts, often with hair follicles about them; but the more delicate elements have degenerated into small islands of debris that stain pink with eosin. Not infrequently a cyst lined by a single layer of cylindrical, or pavement, epithelium is present, more rarely a few acini of cubical cells. The abundant stroma in some measure pushes apart the fragments. For a few more days growth goes on actively and then degeneration becomes dominant.

In an unfavorable individual, on the other hand, grafts show at the end of the second day slight or no cellular connection with the host, and there is absence of vascularization. The great mass of fragments remains lying free. At the fourth day much the same

general picture is presented. There has been no formation of stroma, and degeneration is already far advanced. At the seventh day the fragments are still unorganized, they stain a diffuse pink with eosin, and the necrotic mass is enclosed by a thin zone of fibroblastic tissue from the host. The dead cartilage and squamous epithelium are easily recognizable. At the end of seven days some few living bits of these are yet to be found, which have survived because of their position next the host tissues.

All gradations are encountered between the successful "take" and complete failure. So too it is with tumor-grafts.

In several details the "take" of an embryo graft differs from that of most tumor grafts. The embryonic connective-tissue proliferates actively, whereas that introduced with a bit of carcinoma dies, and its place is taken by cells from the host. In this respect the course of events in the embryo-graft more nearly resembles that in a graft of carcinoma sarcomatodes, since there both epithelial and connective-tissue elements survive transplantation, though, as Haaland[17] showed, dependent for growth on a reaction from the host.

A more important difference has to do with vascularization of the graft. For embryonic tissue this takes place, in part at least, by end-to-end anastomosis of new capillaries from the host with those already present in the tissue introduced, just as happens with Krause's skin graft. In consequence one finds, sometimes after so short a period as twenty-four hours following implantation, sinuses in the embryonic tissue engorged with host's blood. The vascularization of a tumor-graft is much slower because there is no end-to-end anastomosis of vessels. Its occurrence in tumor-grafts has yet to be reported.

This distinction, important as it may appear at first sight does not affect the real issue. For just as every strain of tumor calls forth its own peculiar stroma reaction (Bashford), so may grafted embryonic tissue be considered to. Indeed, it will probably be found that each viable element of this, and every adult, normal tissue capable of growth on transplantation, calls forth what might

[17] M. Haaland, *Third Scientific Report of the Imperial Cancer Research Fund*, 1908, p. 175.

be termed a specific, supporting reaction. Whether vascularization proceed by ingrowth or by direct anastomosis, whether the injected connective-tissue lives or dies, a reaction from the host may be considered necessary for growth of a graft. This is true of implanted embryo and of implanted tumor. Natural resistance of the host is expressed in both instances, by failure of the reaction to occur.

Interesting corroborative evidence is furnished by a study of grafts in which tumor and embryonic tissue have been mixed. Not only will they grow in intimate association, but where one fails because of lack of reaction from the host, the other, as a rule, is observed to have done so more or less completely, depending on which tissue is more robust in withstanding adverse conditions. This is especially noticeable in partial " takes " of the mixed graft. In those regions of such a graft that show a stroma reaction, tumor and embryo may be found growing excellently side by side; and in the parts where it is absent both have died. Plate XXIX, Fig. 6, and Plate XXX, Fig. 7, of sections from the same nodule illustrate this. Such pictures are not always observed, since some tumors have so infiltrative a tendency, or so avid a growth, as to far outstrip the embryo; whereas others are so delicate, or so slow to gain a foot-hold, that the cells do not withstand the conditions in a mixed graft, and any mass formed is made up wholly of embryonic elements.

The behavior of hashed mouse-embryo in the subcutaneous tissue of rats has an importance is view of the well-known fact that the tumors of white mice do not survive transplantation to white rats, though the cells live for some days, and, indeed, may give rise to small nodules. Russell[18] has studied the histology of these cases, and has pointed out that a vigorous stroma of rat-tissue is formed for the tumor-cells of the mouse but does not prevent their death.

When hashed mouse-embryo is injected into the subcutaneous tissue of white rats a nodule develops within the course of four or five days. This may be small or it may be nearly twice as large as that which would have been obtained after the same period in a favorable mouse. Macroscopically such a nodule consists of a

[18] B. R. G. Russell, *Third Scientific Report of the Imperial Cancer Research Fund*, 1908, p. 341.

tough, pale, translucent tissue through which are scattered small opaque masses. Microscopically these latter are identifiable as bits of embryo, more or less well preserved, and they are embedded in a profuse stroma. While at the seventh day, growth of such embryonic fragments is at its height in the subcutaneous tissue of the mouse, already in the rat most of the fragments are far degenerated. One finds dead epithelium in the form of cysts, showing that growth did take place for a brief period; masses of debris that stain pink with eosin; much cartilage, vacuolated and hydropic, and some few bits that have lived and grown (Plate XXX, Fig. 8). The stroma in which these are embedded is in its origin easily traceable to the host, and it contains none of the engorged sinuses that are a striking feature of the graft in the mouse. There is no end-to-end anastomosis of blood-vessels, and the embryonic connective-tissue, during the brief period it lives, is still distinguishably embryonic. In the abundant stroma formed by the host an occasional foreign-body giant cell may be found in the region of the fragments. Even when the host is a mouse such cells are not infrequently seen, bordering on dead portions of the graft. Mouse embryo, then, like mouse tumor, when injected into the rat, lives for some days and calls forth a profuse connective-tissue reaction.

Russell in his discussion of the course of mouse-tumor grafts in the rat assumes that the stroma is in principle the same as that elicited from mouse-hosts; and he remarks on the strange fact that, whereas in mice the immediate growth or death of the tumor depends on the formation or non-formation of this stroma, in rats the presence of a stroma fails to insure life to the neoplasm.

The fact becomes no longer strange if one looks on the reaction of the rat simply as a reaction to mouse-tissue as such, not as a specific, supporting reaction for the neoplasm. Such an assumption has much in its favor. Although the embryo mass calls forth in the rat a stroma, this differs greatly from that in the mouse, both histologically and in its relationship to the implanted material. I have found that when a series of rats and mice are injected with the same embryo mass, in *all* the rats a stroma reaction takes place, irrespective of whether the introduced fragments live or die; while in mice a stroma-reaction occurs only in certain hosts favorable to

growth of the tissue. It would seem that here the rat is responding to a stimulus more general than that which in the mouse causes stroma-formation. As a further test a very delicate mouse-tumor was injected into rats. In these, an abundant stroma was promptly formed for the degenerating cells, while in mice, injected at the same time, a stroma only exceptionally resulted, and the majority of the grafts died. It is difficult to regard the stroma of the rat in such instances as specifically elicited by the neoplasm,—a neoplasm so delicate that under the far more suitable conditions of the mouse-host it is rarely able to call forth the supporting reaction necessary to growth. That an active mouse-tumor may proliferate for a short period in the rat, and the adjoining reactive connective-tissue adapt itself passively to the new-formed structures cannot be denied. But that in its rôle as a neoplasm it elicits stroma is hardly to be granted in view of the above facts. These indicate that the stroma is a reaction of the rat to mouse-tissue as such, whether it be capable of growth or certain to die at once. Levin[19] has pointed out that the reactive proliferation of the connective tissue of the rat is notably profuse.

In the variability of its fate in different individuals, in it, dependence on a tissue-reaction for growth, and in the events that follow its introduction into rats, mouse-tumor resembles mouse-embryo, a normal tissue capable of growth on transplantation. In so much, at least, that has been often regarded as unique are the phenomena only secondarily characteristic of tumor. Primarily they are tissue phenomena.

Thus far in the present paper comparison of the fate of tumor grafts and embryo grafts has concerned itself mostly with the changes involved in their growth and failure. Some of the factors which lead to these results will now be taken up. It is known that a number of natural characters of the individual,—age race, food, nutrition,—affect his behavior as a host for implanted tumor. Are they active for implanted embryo, and in the same way? Are animals resistant to tumor growth resistant also to embryo growth? And, finally, is the resistance of the individual to the growth of en-

[19] I. Levin, *Jour. of Exper. Med.*, 1908, x, 811.

grafted tumor simply one expression of a resistance to the growth of engrafted tissues in general?

Taking up these matters, I have made numerous observations with implanted embryo, often in parallel with tumor, selecting batches of animals of different ages, nutritive condition, etc. In recognition of the fallacy that pertains in transplantation work to isolated observations, each experiment has been done with a considerable series of mice, and the results have been read in averages. The injections have been quantitative in the subcutaneous tissue of the side. Embryo mass to the amount of 0.02 cubic centimeter produces in favorable individuals a nodule without central necrosis, but larger amounts usually fail to become organized throughout.

For the first two or three days after injection, an embryo graft can be felt through the skin as a soft strand, and when failure to take occurs, this may nevertheless become fairly defined, owing to changes about the dead tissues. But it does not gain in size and disappears progressively. When the graft "takes" a nodule rapidly develops from it, that firm, elastic, oblong, can be rolled beneath the finger. From an initial mass of 0.02 cubic centimeter a nodule 10 by 4 by 3 millimeters in size (or of a bulk six times that injected) may develop within six to eight days. Measurement by palpation tells much as to the success of grafts, though there are many border cases, partial takes for example, or, more rarely, instances of inflammatory proliferation about the dead tissue, in which this method is useless. At best it indicates very roughly the results. A special error is introduced by the bursting of one of the thin-walled sinuses characteristic of the vascularization of embryonic tissue. The hemorrhagic cyst that results feels on palpation much like an actively growing nodule. All these were discarded in casting up the results. Infection of the graft is rarely met with and is usually recognizable on palpation, owing to the induration and skin involvement.

As a rule, the animals were killed on the seventh day after injection, and careful notes made of the condition of the grafts. At this period, the embryonic tissue has nearly reached its limit of growth, and a general retrogression is not far off. Energetic tumors, such as those employed, have already at this time made a

perceptible headway. With a little experience it becomes easy to tell from macroscopic examination the fate of the tissues. For in the case of tumor the little nodule, if living, is translucent and firm; if dead, it is soft and opaque, as is the case with dead embryonic tissue. But if this latter has "taken," the firm, cystic, irregular nodule already described is formed. It contains small cysts, translucent bits of cartilage, recognizable as such macroscopically, and it is held together by a well-defined stroma.

For many intermediate cases a microscopic view alone can settle the fate of the implanted tissue. Sections were, therefore, made of every graft. But intermediate cases proved less important to the interpretation of experiments than those in which outspoken failure or success had occurred. So many small factors influence the fate of the graft, such as, its exact position in the host, the parts of it which are next to the host tissues, the proportion of viable fragments, etc., that a conclusion from results other than the most striking is hardly to be attempted. As in the case of tumor, both the presence of growth and its amount require consideration. But there is the weighty difference between growth of mouse-tumor and growth of mouse embryo that the one goes on and becomes progressive, whereas the other ceases after seven to ten days. It was necessary to autopsy the animals before this distinction had begun to manifest itself.

By three methods, then, the fate of every graft was determined: by palpation through the host's skin, by macroscopic observation, and by sections. For purposes of conciseness the first of these will be largely relied upon in presenting results.

Nutrition of the host.—Among factors which influence the success of transplanted tumor this has been recognized as extremely important. The unfavorable influence of poor nutrition of the host has been a matter of general observation. Moreschi[20] has shown with some exactness that, when the food of inoculated animals is reduced to the minimum compatible with life, tumors develop less frequently and grow more slowly than in controls. Others have noted that in sick animals, presumably as a matter of nourishment, tumors do not "take" or grow well. In using grafts of

[20] C. Moreschi, *Zeit. f. Immunitätsforsch., Orig.*, 1909, ii, 651.

embryonic tissue I have been repeatedly struck with the importance of health and nourishment of the host. The following is an illustration.

Seventeen mice of the same lot, three-fourths grown, were inoculated on one side with 0.02 cubic centimeter of tumor mass, and on the other, with a like amount of embryo mass. The embryos used were 1.4 centimeters long, the tumor an adeno-carcinoma (No. 33), originally propagated from a New York mouse. Within three days after inoculation, seven of the animals were taken with an enteritis, characterized by diarrhoea and emaciation, and on the seventh day one died. The grafts in all were measured by palpation on this day. The figures that follow indicate the approximate *transverse* measurement in millimeters of each strand or oblong nodule, as ascertained by palpation during life.

TABLE II.

Malnutrition.

	Healthy.										Diarrhœa—Emaciation.						
Tumor	3	3	3	3	2	3	3	4	3	6	2	2	1	1	1	2	1[1]
Embryo	4	4	3	3	2	5	4	4	3	4	3	2	1	1	1	2	1

Measurements on seventh day.

[1] Died on seventh day.

It will be seen how much more considerable was the growth of both embryo and tumor in the healthy, well-nourished animals. All were killed on the ninth day. Direct measurement of the grafts at that time yielded a contrast similar to, but not so marked as, that in Table II. On palpation through the skin the *consistence* of the nodule has much to do with its size, and this consistence is determined by the organization or death of the graft. It was found that only in the sick animals had failure of any of the grafts occurred. Microscopically, in three of these both embryo and tumor had failed totally, and in the other four the "takes" had been only partial, as compared with excellent "takes" in all of the healthy mice. No evidence of infection of the grafts was seen, either macroscopically or histologically. The age of the animals was especially favorable to tumor growth, and, as will later be

shown, to embryo growth also. The observation given is but one among many. In thin mice without obvious illness it is notably the rule for embryo-grafts to have poor success. The nutritive condition of the host influences very greatly the grafts' fate; and in experiments this factor is always to be reckoned with. Only when animals are in the same general body state, are fed the same food, and are kept under identical conditions, can results with them be trusted.

The question of nutrition in its immediate relation to the grafts has been dealt with to some extent in a discussion of the fate of mixtures of tumor and embryo. It has been pointed out that in such a mixed graft both tissues have a tendency to grow or fail at the same situations. This suggests that they require much the same immediate nutritive conditions.

Age of the Host.—Bashford[21] was the first to show that mice not yet grown are more favorable than adults as hosts for implanted tumor; and his observations have been abundantly confirmed. It is now customary in the routine transplantation of tumor to use animals one-half to three-fourths grown in order to obtain the favoring influence of youth without the large mortality incident to it at earlier periods. The following experiments demonstrate that embryo grafts succeed better in such hosts than in adults.

EXPERIMENT I.—Eight adult white mice and eleven three-fourths grown were injected with 0.02 cubic centimeter each of embryo mass (embryos 1.4 centimeters long). Measurements by palpation were made on the seventh day and the animals killed and examined. Table III gives, in millimeters, the approximate transverse measurement of the nodules, as obtained on palpation.

TABLE III.

Grafts in Old and Young Mice.

	Grafts at seventh day.										
Eight Adults	2	2	1	2	3	1	3	1			
Eleven Young	3	3	3	3	3	2	3	3	3	2	2

At autopsy all of the grafts in the young were found as firm, translucent nodules. In three of the eight adults similar nodules were present, but in the other five most of the embryonic tissue was opaque and dead.

[21] E. F. Bashford, J. A. Murray, M. Haaland and W. H. Bowen, *Third Scientific Report of the Imperial Cancer Research Fund*, 1900, p. 265.

EXPERIMENT II.—Seven adult mice and five three-quarters grown were given each 0.03 cubic centimeter of embryo hash subcutaneously. The embryos for this were 2.2 centimeters in length. The transverse measurements of the grafts in millimeters, as obtained by palpation at the tenth day, are given in Table IV.

TABLE IV.

Grafts in Old and Young Mice.

	Grafts at tenth day.						
Seven Adults	3	2	2	2	2	3	2
Five Young	4	3	4	3	3		

These animals were allowed to live for nineteen days, and it was found at autopsy that retrogression had gone so far in the grafts, that there was small choice between them. The youth of some of the hosts had not retarded retrogression.

EXPERIMENT III.—Three half-grown white mice and eight adults were killed on the eighth day after an injection into each of 0.04 cubic centimeter of embryo hash (embryos 1.5 centimeters long). This amount was so large as to make difficult its complete organization; and during life little difference in size of the grafts was noted.

Autopsy showed in the three young mice, healthy, cystic, largely cartilaginous, nodules, held together by a delicate stroma; whereas in two of the eight adults the embryonic tissue was dead, in five others, there had been some growth, and in one only had a good "take" occurred.

Whether extreme youth of the host is a condition favorable to the growth of tumors is yet unsettled. In Bashford's experiments no animals less than two weeks old were employed.[22] Féré[23] found that implanted embryo did not succeed as well in chicks as in adult fowls. I have carried out experiments with both embryo and tumor, using mice one to three days old. The results will be detailed in a separate paper. They would seem to indicate that these very young animals are unfavorable as hosts for embryo, while for tumor, if it succeed in obtaining a foot-hold, they show themselves especially suitable. The question presented is probably one of relative "avidity" of the implanted and host tissues.

Race of the Host.—The fate of embryo grafts introduced into animals of a different species (mouse-embryo into rats) has been demonstrated to be in essential the same as that of tumor-grafts.

[22] E. F. Bashford, J. A. Murray and M. Haaland, *Third Scientific Report of the Imperial Cancer Research Fund*, 1908, p. 359.

[23] C. Féré, *loc. cit.*

To test whether the race of the host influences the success of embryo-grafts as it does that of tumor, I have transplanted material from white mice into both whites and blacks of the same approximate age and nutritive condition. While it does not follow that resistance to tumor varies with the color of an animal's coat, or with other external characteristics,[24] yet it may be taken as a general law that a tumor is most easily transplanted to animals of the same variety in which it appeared. This can be tested for embryo, though that is hardly necessary in view of facts with regard to the grafting of normal adult tissues, showing that these respond best to auto-transplantation, less well to iso-transplantation, and poorly, if at all, to hetero-transplantation.

EXPERIMENT I.—Nine black and ten white mice of unknown parentage were injected subcutaneously with 0.04 cubic centimeter each of a mass made of embryos from a white female. These embryos were 1.4 centimeters in length. Owing to the fact that the skin of the black mice was thicker than that of the whites, measurement on palpation proved fallacious.

Autopsy of all was performed on the eighth day, and the results as here given are from macroscopic and microscopic findings. They have been arranged in the order easiest for comparison.

TABLE V.

Grafts in Black and White Mice.

	White mice.	Black mice.
Adults	Excellent growth.	Fair growth.
	Fair growth.	Slight growth.
	Fair growth.	Slight growth.
	Fair growth.	Graft dead.
	Slight growth.	Graft dead.
	Graft dead.	Graft dead.
	Graft dead.	Graft dead.
Young (¾ grown)	Excellent growth.	Good growth.
	Excellent growth.	Fair growth.
	Excellent growth.	

EXPERIMENT II.—Sixteen black and fourteen white mice of unknown parentage were used in this experiment. The material for injection came from a white mouse, and consisted of embryos 1.1 centimeters in length, decapitated and eviscerated. Of this, 0.02 cubic centimeter was implanted in each animal. Autopsy was done at the seventh day. The results are from the macroscopic and microscopic examinations.

[24] E. Tyzzer, *Jour. of Med. Research*, 1909, xxi, 519.

TABLE VI.

Grafts in Black and White Mice.

	White mice.	Black mice
Adults	Excellent growth.	Excellent growth.
	Excellent growth.	Good growth.
	Very good growth.	Good growth.
	Very good growth.	Fair growth.
	Good growth.	Fair growth.
	Good growth.	Graft dead.
	Slight growth.	Graft dead.
	Graft dead.	Graft dead.
	Graft dead.	Graft dead.
		Graft dead.
Young (¾ grown)	Excellent growth.	Excellent growth.
	Excellent growth.	Excellent growth.
	Excellent growth.	Good growth.
	Good growth.	Fair growth.
		Fair growth.
		Slight growth.

While the black animals proved somewhat unfavorable as hosts for the embryo mass, there were striking exceptions. In the young individuals, growth was good,—illustrating again the importance of age of the host; and in occasional adults the graft developed well. So, too, it is with transplantable tumors. The immunity based on race, age, or other natural factors like that acquired from inoculation, is seldom absolute. There occur individuals which, acting against the rule, are still susceptible. The cause for this is unknown.

Sex.—The sex of the host does not influence the fate of transplanted tumor nor that of transplanted embryo.

So far as the foregoing factors determine the character of a host, it may be said that the question as to whether the behavior of the individual to implanted embryo and implanted tumor is relatively the same has been answered in the affirmative. Tumor tissue is probably much the more delicate as an indicator of peculiarities in the host, by reason of the opportunities afforded through its prolonged growth.

There remain untested important influences. Haaland[25] and

[25] M. Haaland, *Münchener med. Woch.*, 1907, xliv, 713.

Nègre[26] have shown that changes in food will alter an animal's status as a potential host for tumor. Pregnancy is known to exert an influence.[27] I have made large numbers of parallel observations with tumor and embryo on animals in which these factors were, so far as possible, controlled. But the tissues used in parallel were obtained from different individuals, came from a different soil, so to speak, and this must necessarily have affected the results. While it seemed that in general the new host's influence was similar on both grafts yet this has not been fully demonstrated.

Acquired Immunity.—The conditions which determine a host's natural resistance have been discussed. But it is well known that an immunity against implanted tumor can be induced. Is it possible to bring about a similar immunity to implanted normal tissue?

Acquired resistance to implanted tumor manifests itself in the same way as the natural, that is, by the non-appearance of a supporting stroma for the graft.[28] Distinct evidence of cytotoxic action on the introduced tissue is lacking; and at present there is little reason to believe that the immunity is cytotoxic in character. Indeed it cannot be referred to any class hitherto known. Bridré[29] and Bashford, Murray and Haaland consider it a tissue immunity.

A method which has commended itself for the production of such immunity is that of inoculation into susceptible animals of tumor-cells, living, but weakened in some way. I have been struck with the degree of protection brought about by an intraperitoneal inoculation of a weak suspension of tumor material in normal salt solution. With a suspension of embryonic material the method has been used in the following attempts to produce an immunity to embryonic tissue.

Experiment I.—Eleven adult mice were given intraperitoneally 1 cubic centimeter each of a thick suspension of mouse-embryo in normal salt solutoin, as obtained by rubbing up the material in a mortar and filtering through sterile gauze. These embryos were 1.8 centimeters long. Ten days later the animals were inoculated subcutaneously with 0.02 cubic centimeter each of a mass made from

[26] L. Nègre, *Compt. rend. Soc. de biol.*, 1909, lxvii, 28.

[27] M. Haaland, *loc. cit.;* L. Cuénot and L. Mercier, *Compt. rend. Soc. de biol.*, 1909, lxvii, 736.

[28] B. R. G. Russell, *loc. cit.*

[29] Bridré, *Ann. de l'Inst. Pasteur.*, 1907, xxi, 760.

embryos 1.2 centimeters in length. Seven controls of the same size and age were also inoculated. The results on palpation at the seventh day follow. The approximate transverse measurement of the nodules, in millimeters, is given.

TABLE VII.

Immunity.

	Graft at seventh day.
Eleven Emulsion Mice.....	1 2 1 1 + 2 0 0 + 1 +
Seven Controls	2 1 + 1 3 + 3

All the animals were autopsied on the eighth day. In one that had received the suspension there was a fair "take"; in two, a partial "take"; and in the remaining eight all the embryonic tissue was dead. Of the controls five showed an excellent "take," and in the remaining two the graft was dead.

EXPERIMENT II.—Fifteen mice three-fourths grown, were injected with suspension as in Experiment I, and reinjected eleven days later. For the first suspension embryos 1.5 centimeters in length were employed; for the last others of the same size. Ten days after this second treatment all were given subcutaneously 0.02 cubic centimeter of embryo mass (embryos 1.5 centimeters long) and seventeen controls of the same age and lot received a like dose. The results on palpation and on autopsy at the seventh day follow. The approximate transverse measurement in millimeters of the oblong nodules is given.

TABLE VIII.

Immunity.

Graft at seventh day.	Take.	Partial Take.	Failure.
Fifteen Emulsion Mice.			
1 1 + 2 1 1 1 + + 1 1 1 1 +	0	6	8
Seventeen Controls.			
1 1 1 3 2 3 2 1 1 2 3 1 2 2 + 1 2	9	7	1

EXPERIMENT III.—Ten mice, one-half to three-fourths grown, were injected with a thick suspension of embryonic tissue. For this embryos 2.2 centimeters in length were employed. There were also injected seven control animals of the same size and lot with 1.0 cubic centimeter of 0.9 per cent. sodium chloride solution. Ten days later all were implanted with 0.02 cubic centimeter of embryo mass in the subcutaneous tissue of each side. Embryos 1.1 centimeters in length went to make this mass.

Palpation during life showed that the grafts grew better in the control animals. Autopsy was done on the seventh day. The results are presented in Table I, "Fate of Grafts A and B in the Same Animal." In five of the control animals (Column II) good growth had occurred; in one, hemorrhage had taken place; in another the grafts were dead: whereas of the ten immunized mice (Column I) two only showed good growth of the grafts, one fair, one slight; in one hemorrhage had occurred and in five the graft was dead.

The mice of these last two experiments were at an age most favorable to embryo growth.

It is evident that resistance to the growth of implanted embryo can be brought about by a method similar to that used for tumor. Furthermore, a microscopical examination has shown that this acquired resistance to implanted embryo manifests itself in the same way as that to tumor, namely, by failure of the host tissues to furnish to the graft a supporting stroma. But the immunity is not absolute: in some of the treated animals growth takes place. This has been noted of tumor by others.

Since the completion of the above experiments a paper has appeared by Fichera,[30] detailing the production by another method of immunity to transplanted embryo. Fichera made successive implantations of rat embryo in rat hosts, waiting always till the nodule of one implantation had disappeared before implanting anew. Thus he found that, while growth and retrogression of the first graft required from six to eight months, for the fifth, it took but twenty to thirty days, although in controls the same material grew and persisted for months. It is to be noted that Fichera ignored the effect of age of the host, which probably had some importance, in view of the fact that his inoculations into each individual required about two years, no inconsiderable period in the life of a rat. But the striking differences between the results in the injected rats and in the controls are hardly to be explained through differences in age alone, since white rats, old and young, make remarkably good hosts for implanted embryo.[31]

Cross-Immunity.—No attempts have been made to produce immunity to implanted normal tissue by injections of tumor. But Schöne[32] has demonstrated that immunity against tumor can be produced with embryo, and Bashford[33] and others that it can be elicited with adult tissues. These with the foregoing observations link together more closely tumor-immunity and embryo-immunity. The two are not only similar in principle but would seem to be individual manifestations of a more general tissue immunity.

[30] G. Fichera, *loc. cit.*

[31] M. Askanazy, *loc. cit.*

[32] G. Schöne, *Münchener med. Woch.*, 1906, liii, 2517.

[33] E. F. Bashford, J. A. Murray and W. Cramer, *Proc. of the Royal Soc., Series B.*, 1907, lxxix, 164.

SUMMARY.

The present paper deals with a comparison of the conditions which determine the fate of transplanted tumor and of a transplanted normal tissue capable of growth. Mouse embryo and mouse-tumor were employed as material. It was shown that individuals differ as hosts for transplanted embryo, some being naturally resistant to its growth, and some favorable, just as is known to be the case where tumor is concerned. The fate of implanted tumor depends directly on whether it elicits from the host a vascularizing stroma. So, too, it is with implanted embryo. Furthermore mouse-embryo, like mouse-tumor, when introduced into rats calls forth a stroma and grows for a brief period.

In attempt to answer the question as to whether individuals favorable (or resistant) to implanted tumor are likewise favorable (or resistant) to implanted embryo, it was shown that the factors of age, nutritive condition, and race, which are potent in determining an animal's status as a tumor-host, act similarly in determining that for embryo.

Using embryonic tissue and a method which has proven effective for the production of immunity to implanted tumor, an immunity to implanted embryo was brought about. This immunity manifests itself in the same way as that for implanted tumor, namely, by an absence of the stroma-reaction necessary to life of the engrafted tissue.

These results demonstrate how largely tumor obeys in its adaptation to a new host and growth therein, the general laws regulating a transplanted normal tissue. Besides the phenomena here dealt with many others that have held the attention of workers with transplantable tumors are probably not peculiar to neoplasm. The present findings emphasize the importance of the tumor-problem as a tissue-problem; and they further indicate how essential it is in cancer work to discriminate between characters unique with tumor and those which it possesses in common with normal tissue.

EXPLANATION OF PLATES.

PLATE XXVII.

FIG. 1. Injected embryo graft. The injection (india ink) through the aorta of the host. Hematoxylin and eosin.

FIG. 2. Embryo graft. Typical picture of a successful graft (seven days after implantation). Hematoxylin and eosin.

PLATE XXVIII.

FIG. 3. Tumor graft. Typical picture of a successful graft (seven days after implantation). The tumor is an adeno-carcinoma of rapid growth. Hematoxylin and eosin.

FIG. 4. Failure of an embryo graft (six days after implantation). Hematoxylin and eosin.

PLATE XXIX.

FIG. 5. Failure of a tumor graft (seven days after implantation). Hematoxylin and eosin.

FIG. 6. Graft of mixed tumor and embryo (seven days after implantation). From same nodule as Plate XXX, Fig. 7. Tumor and embryo, side by side, have failed to "take." At the margin of the graft a few living cells of both persist. Hematoxylin and eosin.

PLATE XXX.

FIG. 7. Graft and mixed tumor and embryo. From same nodule as Plate XXIX, Fig. 6. Tumor and embryo are growing, side by side. Hematoxylin and eosin.

FIG. 8. Mouse embryo in the rat (seven days after implantation). Hematoxylin and eosin.

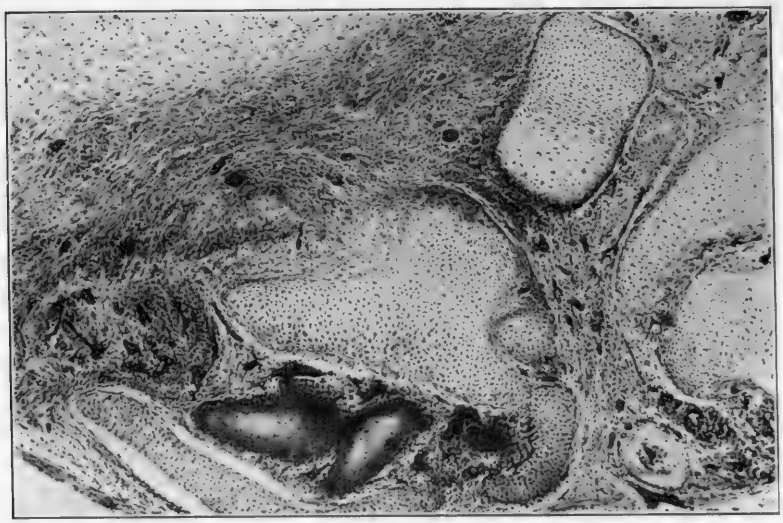

FIG. 1.

FIG. 2.

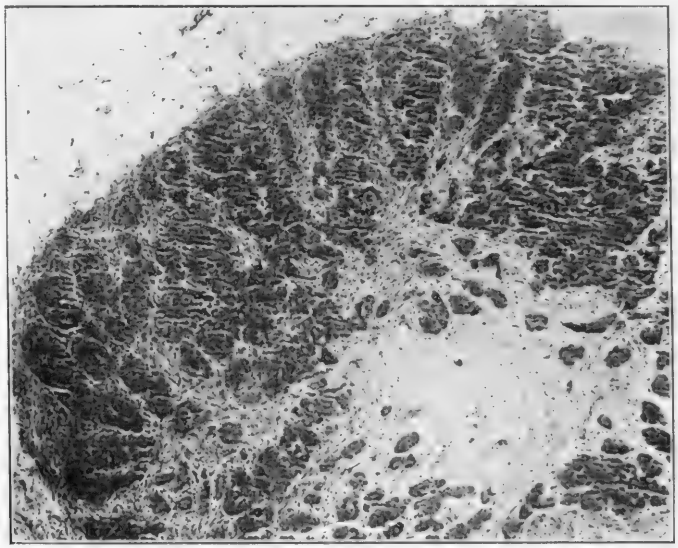

FIG. 3.

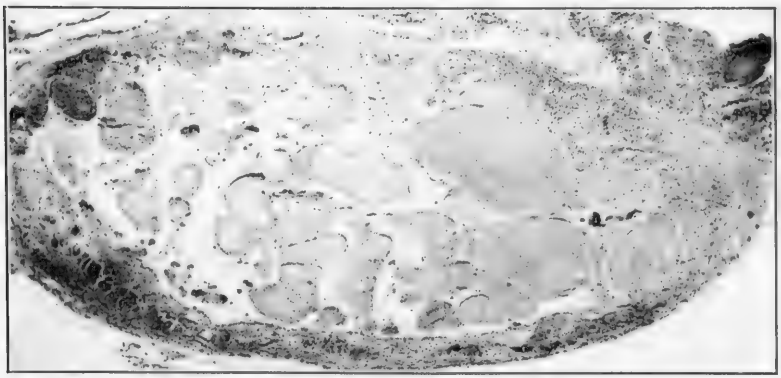

FIG. 4.

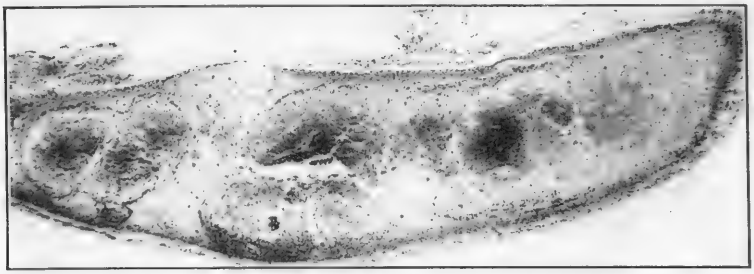

FIG. 5.

FIG. 6.

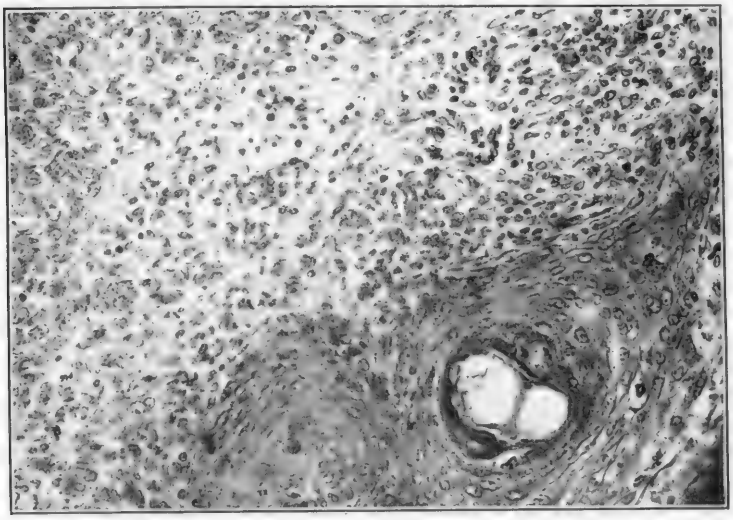

FIG. 7.

FIG. 8.

ON THE RELATION OF COMBINED INTOXICATION AND BACTERIAL INFECTION TO NECROSIS OF THE LIVER, ACUTE YELLOW ATROPHY AND CIRRHOSIS.[1]

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Plates XXXI–XXXVIII.

Experiments have shown that various toxic substances administered to animals produce degenerative changes in the liver and, when this administration is prolonged during a considerable period, increase of the interstitial tissue follows. Nevertheless the results of such experiments have been inconstant and the lesion which has been obtained has rarely possessed the clinical and anatomical characters of the cirrhosis common in man. In the attempt to produce cirrhosis alcohol has been repeatedly employed because it is believed to be the common cause of hepatic disease; phosphorus has been employed because it quickly produces advanced degenerative changes both in man and in animals.

Nevertheless a clear knowledge of the conditions upon which the occurrence of cirrhosis depends has not been obtained. Alcohol has been regarded as the most frequent cause of the disease, yet, on the one hand, it is well known that a history of excessive use of alcohol is obtained in little more than 50 per cent. of instances of cirrhosis; that the disease occurs in children some of whom have had no opportunity to use alcohol in any form. On the other hand, cirrhosis occurs in only a small proportion of those who are addicted to the use of alcoholic drinks; it was present in only 5 or 6 per cent. of the alcoholics examined by Baumgarten.[2] Degenera-

[1] Presented before the Philadelphia Pathological Society, March 10, 1910.

[2] *Verhandl. d. deutschen path. Gesellsch.*, 1908, xi, 229.

tive changes in the liver indicated by infiltration with fat is more common.

Among the large number of those who have tested the effect of long-continued administration of alcohol upon animals a few have claimed that they have been able to produce increase of the interstitial tissue of the liver. Straus and Blocq[3] found the portal spaces infiltrated with cells and in some instances noted an increase of connective tissue at the periphery of the hepatic lobule. Mertens[4] allowed rabbits to breathe an atmosphere saturated with alcoholic vapor during periods as long as eleven months and twenty-eight days. In one group of animals which died after a few months there were degenerative changes in the parenchymatous cells and although there was no increase of connective tissues, ascites was present; in a second group of animals which survived a longer period of administration, new interstitial tissue was found about the portal spaces and in certain instances extended from portal spaces to sublobular veins. These experiments were made upon rabbits; the spontaneous occurrence of cellular infiltration and of periportal cirrhosis referable to coccidia or other parasites is not infrequent but does not explain all of the changes which have been found. Afanassijew[5] gave mixtures of ethyl and amyl alcohol to dogs, rabbits and guinea-pigs but failed to cause cirrhosis; v. Kahlden[6] used various animals, administering alcohol during periods up to 243 days with like result. Friedenwald[7] found increase of interstitial tissue in some animals (rabbits), but in others which had received from five to eight cubic centimeters of alcohol during four years he observed no trace of cirrhosis. Other negative experiments might be cited; simple administration of ethyl alcohol fails to increase the interstitial tissue of the liver. Moreover, the production of changes indicating alteration of the hepatic cells has been inconstant and often trivial.

With the purpose of establishing a definite relation between intense degeneration of hepatic cells on the one hand, and new forma-

[3] *Arch. de physiol.*, 1887, Series 3, x, 409.
[4] *Arch. internat. de pharmacod.*, 1896, ii, 127.
[5] *Ziegler's Beit.*, 1890, viii, 443.
[6] *Ibid.*, 1891, ix, 349.
[7] *Jour. of the American Med. Assn.*, 1905, xlv, 780.

tion of fibrous tissue on the other, many attempts have been made to produce cirrhosis by continued administration of phosphorus. Acute poisoning with phosphorus, it is well known, is associated with intense fatty degeneration of the liver cells associated with some necrosis. Several observers, Afrecht,[8] Krönig,[9] Ackermann[10] and Dinkler[11] have produced increase of interstitial tissue by repeated administration of small quantities of this poison. Ziegler and Oblonski failed to produce changes resembling cirrhosis of man, but Aufrecht[12] by prolonged administration has produced a nodular liver which, he claims, exhibits the usual characters of human cirrhosis. New formation of fibrous tissue has its origin at the periphery of the lobule in contact with the portal spaces.

By means of hemagglutinative serum prepared by immunization of rabbits with the red blood corpuscles of the dog, Pearce[13] has produced in the dog agglutination of corpuscles within the hepatic capillaries and consequent irregularly distributed necrosis of hepatic parenchyma. The necrotic issue is rapidly absorbed and replaced by connective tissue. Liver cells outside of the dead area undergo multiplication and newly formed structures resembling bile ducts penetrate the new interstitial tissue.

The prevailing view that proliferation of connective tissue is the consequence of injury and destruction of the parenchymatous cells of an organ has caused a few experimenters to administer chloroform with the purpose of reproducing human cirrhosis. The toxic effect of chloroform upon the liver is well known. The early observers have been doubtful whether the resulting lesion is intense fatty degeneration or necrosis, but subsequent study has shown that both may be caused by chloroform. The recent experiments of Howland and Richards[14] and of Whipple and Sperry[15] in this country have shown that anesthesia by chloroform continued from

[8] *Deutsches Arch. f. klin. Med.*, 1879, xxiii, 331.
[9] *Virchow's Arch.*, 1887, cx, 502.
[10] *Virchow's Arch.*, 1880, lxxv, 396.
[11] Inaug. Diss., Halle, 1887.
[12] *Deutsches Arch. f. klin. Med.*, 1897, lviii, 302.
[13] *Jour. of Exper. Med.*, 1906, viii, 64; *Jour. of Med. Research*, 1906, xv, 99.
[14] *Jour. of Exper. Med.*, 1909, xi, 344.
[15] *Bull. of the Johns Hopkins Hosp.*, 1909, xx, 278.

one to three hours and repeated on two or three successive days is followed by hyaline necrosis of the columns of liver cells occupying perhaps the central two-fifths of each lobule; the liver cells in immediate contact with the portal spaces alone remain intact and occasionally the entire hepatic substance save a narrow fringe of cells about each portal space is destroyed. Fatty degeneration accompanies necrosis and occurs where the action of the poison is not sufficiently intense to produce cell death. With mild poisoning the center of each lobule exhibits fatty degeneration instead of necrosis and in some instances central fatty degeneration and necrosis occur in the same liver.

The extensive necrosis of the liver caused by chloroform offers an opportunity to test the relation of cirrhosis to degenerative changes in the hepatic parenchyma; to determine to what extent necrotic parenchymatous cells are replaced by fibrous tissue. The experiments of Whipple and Sperry are particularly instructive. Anesthesia with chloroform, for example, during three hours on one day or during two and a half hours on three successive days, has been followed by hyaline necrosis of the central three-fifths of each hepatic lobule; the extent of necrosis has been determined by removal of a bit of liver tissue on the day following the last anesthesia. The animal recovers completely and examinations made at the end of one, two and three weeks have shown that repair is almost complete after two weeks, whereas at the end of three weeks the liver has returned to normal. Mononuclear phagocytic cells invade the area of hyaline necrosis and ingest particles of dead hepatic cells. Mitotic nuclear figures within the surviving hepatic cells show that regeneration is in progress and columns of liver cells elongate to replace those which have been destroyed. There is no new formation of fibrous tissue and no cirrhosis follows destruction of more than half of the entire organ.

Nevertheless, the literature of the subject contains records of experiments in which cirrhosis of the liver has been produced by chloroform. Mertens (1896) has produced cirrhosis in rabbits by daily subcutaneous injection of small quantities of chloroform (0.1 to 0.2 cubic centimeters) dissolved in liquid paraffin, continued in some instances as long as six months. The liver becomes pale and

hard and in some instances has the nodular appearance of atrophic cirrhosis. The parenchymatous cells undergo various forms of degeneration and bands of newly formed fibrous tissue traverse the organ. According to Mertens the new bands of fibrous tissue have their origin about the portal spaces at the periphery of the lobules but later unite neighboring portal spaces and are continuous with the tissue about the hepatic veins. Herter and Williams[16] have produced cirrhosis in dogs by similar means. After inhalation of chloroform during a period from an hour to an hour and a half, on twenty-four occasions within eight weeks, the liver contained abundant cellular connective tissue between and within the lobules; bile ducts had undergone proliferation and fatty and hyaline liver cells were abundant. Two other experiments had a similar result.

The experiments of Whipple and Sperry, who found complete restoration to normal after extensive hyaline necrosis, have suggested that recovery occurs because regeneration of parenchyma is active and uninterrupted. Numerous experiments have shown that chloroform administered by stomach has the same effect as when administered by inhalation. A dose of from 0.5 to 1 cubic centimeter per kilogram of body weight repeated on three successive days usually produces well-marked necrosis of the central part of the lobule. After an interval of three days, necrotic cells are in large part removed, the central part of the lobule is infiltrated with numerous cells, in part mononuclear phagocytes, and healing is in progress. This process may be interrupted by a further administration of chloroform repeated, as before, on successive days. Such continued insults to the organ, repeatedly producing grave degenerative change, are followed by sclerosis, by regenerative change indicated by proliferation of parenchymatous cells and by abundant new formation of structures resembling bile ducts.

The following experiment illustrates the sclerosis which may occur within the short period of two months.

A dog (Chlor. i) weighing 6,250 grm. has been given 1 c.c. per kilo on three successive days, followed by a rest of three days, in turn followed by chloroform so that it has received in all 218 c.c. of chloroform. It has become slightly jaundiced and the superficial abdominal veins have been considerably dilated. At autopsy the veins of the portal system are dilated, the tissue about

[16] *Proc. of the Soc. for Exper. Biol. and Med.*, 1905, iii, 23.

the pancreas is œdematous and the peritoneal cavity contains a small quantity (6 c.c.) of fluid. The liver is small (weighing 185.5 grm. = 1/33 of original body weight), yellow and resistant to the knife.

Examination has shown that about one-third of the sectional area consists of very cellular, newly formed connective tissue (Plate XXXI, Fig. 1) situated about the central veins and in contact with the sublobular vessels. The liver cells are in columns which radiate from the portal spaces; they have the appearance of regenerating hepatic parenchyma, the columns being formed by regularly disposed cells in double row with the nuclei regularly arranged along either margin of the column. Mitotic figures occur but they are not numerous. Near the center of lobules within the new fibrillated tissue are so-called newly formed bile ducts composed of cubical cells forming well-defined tubules.

Increase of fibrous tissue has been associated with portal obstruction and serous transudation. The newly formed fibrous tissue occupies that part of the lobule which is the site of necrosis caused by chloroform. Active regeneration of parenchyma and new formation of bile ducts having no relation to those in the portal spaces has occurred.

A second experiment is noteworthy because the same poison administered by a somewhat different method has produced a wholly different lesion.

A large animal (Chlor. iv) weighing 10,100 grm. has received 2 c.c. of chloroform per kilo on three successive days; a rest of six days has been followed by chloroform repeated as before; the animal has died at the end of sixty days after it has received 424 c.c. of chloroform. The liver is fairly large (weighing 394 grm. = 1/26 of original body weight) with superficial indentation corresponding to the lobulation, and has a bright canary-yellow color.

Thick bands of cellular fibrous tissue traverse the organ; they are usually in the center of the lobule but their position is often obscure. The parenchyma is the site of intense fatty degeneration, almost every cell being swollen and distended with fat. Newly formed bile ducts are rarely found. The lesion reproduces with fair accuracy the cirrhosis with fatty degeneration which is believed to occur as the result of excessive drinking of beer.

In another experiment which may be cited, the animal has lived longer.

A dog (Chlor. iii) weighing 9,150 grams has lived three months and has received approximately 32 doses of one cubic centimeter per kilo (=393.5 c.c. chloroform). There has been jaundice and dilation of superficial abdominal veins. Veins of the portal system are dilated and the peritoneal cavity contains serous bile-stained fluid (14.5 cubic centimeters). The liver (weighing 422 grams = 1/22 of original body weight) is pale yellow.

Whereas fibrous tissue in proportion to sectional area is not so abundant as in preceding experiments, it is denser and poorer in cells. The relation of newly formed tissue to central veins is apparent but less conspicuous than in the other experiments, for the normal lobulation is much distorted. In some places patches and bands of fibrous tissue may be in contact with portal spaces. Small islands of parenchyma may be completely surrounded by fibrous tissue, and in many places, fine bands of new tissue penetrate between columns of liver cells.

The lesion which has been described begins in the center of the lobule and surrounds the central vein; the relationship between new formation of fibrous tissue and necrosis of parenchyma is obvious. Nevertheless, long continued recurring injury with destruction and regeneration of parenchyma tends to distort the normal architecture of the gland so that lobulation becomes obscured. Advanced cirrhosis has occurred with considerable rapidity and degeneration of parenchyma has been much more conspicuous than regeneration. The regenerative changes which, associated with contraction of the newly formed fibrous tissue, give to the liver of atrophic cirrhosis its nodular appearance have not been produced, and the liver in all of these experiments has been fairly smooth with at most fine granulation corresponding to the disposition of the lobules.

It has long been known that the liver bears a peculiar relation to bacterial infection. The endothelial cells lining the capillaries of the liver and the neighboring stellate cells are capable of active phagocytosis, and as Wyssokowitch[17] found more than twenty-five years ago, take up bacteria injected into the circulating blood. Experiments of Welch and Blachstein[18] showed that *Bacillus coli* and typhoid bacillus injected into the vein of a rabbit appear in the bile

[17] *Zeit. f. Hyg.*, 1886, i, 1.

[18] *Bull. of the Johns Hopkins Hospital*, 1891, ii, 96.

and may persist for weeks in the gall bladder. Numerous experiments have subsequently confirmed these observations. Experiments of Buxton and Torrey[19] show that typhoid bacilli injected into the peritoneal cavity quickly enter the blood and are deposited in various organs. At the end of an hour the number in the blood is small; plating on agar shows that the number which accumulates in the liver is far greater than that in other organs.

Central necrosis implicating from half to three-fifths or even more of each lobule and identical with that produced by chloroform is not infrequently found in the liver of individuals dying with a considerable variety of conditions. Mallory found the lesion 95 times among 1,190 autopsies, usually in association with acute bacterial infection, such as acute endocarditis, lobar pneumonia, peritonitis, diphtheria, etc. In approximately one-half of the cases in which there was bacteriological examination, *Streptococcus pyogenes* was isolated; other microörganisms, such as *Diplococcus lanceolatus, Bacillus diphtheriæ* and *Staphylococcus aureus* have been found. Mallory has expressed the opinion that the lesion is due to the presence of a circulating toxin, most frequently derived from the streptococcus.

Several years ago, I noted a remarkable lesion occurring in the liver of a child who had died with general peritonitis following gonorrheal infection.[20] A conspicuous circle of hyaline necrosis occurred in each lobule in a zone separated by living cells both from the central vein and from the portal spaces. Examining the livers from nearly 1,000 autopsies, I found 14 examples of this lesion which may be termed mid-zonal necrosis. The lesion is accompanied by fatty degeneration of the hepatic cells in the center of the lobule and occasionally of the cells at the periphery of the lobule in contact with the portal spaces as well. The lesion has been associated with acute suppurative conditions (general peritonitis in 6 instances, operative or ulcerative lesions of the gastro-intestinal tract in 3, general pyemic infection in 3, etc.), and in 11 instances bacteriological examination has shown the presence of staphylococcus (in 6), *Bacillus coli* (in 3 alone, in 2 combined with

[19] *Jour. of Med. Research,* 1906, xv, 18.
[20] *Jour. of Med. Research,* 1904, xii, 147.

streptococcus) or *Bacillus proteus vulgaris* (in 2). The infrequency of the lesion and the frequency of severe bacterial infection indicate that the association of some undetermined factor, perhaps some disturbance of metabolism, is essential for its production.

The experimental reproduction of this peculiar lesion will serve to show that the effect of bacterial infection may be profoundly modified by the presence of a toxic substance which has entered the blood.

A large dog (Chlor. xviii) weighing 15,200 grm. has been given 7.6 c.c. of chloroform (0.5 c.c. per kilo) on four successive days; on the fifth day, 0.5 c.c. of a 24-hour bouillon culture of *Bacillus coli* has been injected into the jugular vein. On the eighth day, chloroform has been again administered. The animal has been killed on the following day. The liver is large and deep red; after blood has partially escaped from the tissue, the centers of the lobules are yellow. Lymphatics upon the surface of the gall bladder are conspicuously dilated; the bile contains *Bacillus coli* in pure culture.

The central part of each lobule, occupying one-half the space from central vein to portal space, exhibits fatty degeneration, the cells being filled with fine vacuoles. At the periphery of the lobule liver cells are well preserved and contain only a few fat droplets. In a conspicuous but narrow intermediate zone, forming a circle within each lobule, liver cells have undergone hyaline necrosis, have lost their nuclei, and stain bright pink with eosin.

It is noteworthy that the same peculiar mid-zonal necrosis may be reproduced experimentally by another poison, namely, phosphorus in combination with another bacterium, *Streptococcus pyogenes,* in another animal, the rabbit. The following experiment illustrates very clearly the susceptibility of injured liver to bacterial infection.

Four animals (Rabbits Phos. 2, 3, 5 and 6) received 1/50 grain of phosphorus by mouth daily; during several days administration was suspended so that each animal received during 24 days 14/50 grains. Two of these animals were repeatedly inoculated in the ear vein with *Streptococcus pyogenes;* these animals died on the same day, 24 days after the beginning of the experiment and the two animals which received phosphorus alone were killed.

Save for trivial fatty degeneration the livers of the two animals (Rabbits Phos. 2 and 3) which received phosphorus alone exhibit no noteworthy change (Figs. 3 and 5). Those which have received

streptococcus exhibit advanced degenerative change, causing injury or destruction of the entire hepatic parenchyma. In one (Rabbit Phos. 6), in which the changes are more profound, there is diffuse fatty degeneration accompanied by hyaline necrosis, and hemorrhage in the center of the lobules (Fig. 4). In the second infected animal (Rabbit Phos. 5), the center of each lobule exhibits fatty degeneration; about this central fatty tissue is a conspicuous ring of hyaline necrotic cells which separates the fatty cells in the center from a relatively normal peripheral zone in contact with the portal spaces (Fig. 2). This lesion which is produced neither by the poison nor by the bacterium acting alone demonstrates the peculiar effect of bacterial infection upon an injured liver.

The effect of hepatic injury upon the progress of bacterial infection has been little studied. Adami[21] in America and Hanot[22] and others in France have maintained that cirrhosis of the liver may be referable to chronic bacterial infection. The experimental production of cirrhosis by administration of toxic substances such as chloroform and phosphorus proves that introduction of bacteria is not essential. Repeated inoculations with various bacteria usually fail to increase the interstitial tissue of the liver; some observers have found increase of the interstitial tissue of the liver after injection of various microörganisms (Scagliosi,[23] Dantschatoff-Grigorevski,[24] Bjorksten[25]), but the change has usually been associated with suppurative cholangitis, and increase of interstitial tissue has occurred about the portal spaces. Bacteria capable of causing cirrhosis in animals have been isolated by Weaver[26] and by Hektoen.[27]

In a series of experiments, I have attempted to modify the effect of chloroform by accompanying injection of certain bacteria. By

[21] *British Med. Jour.*, 1898, ii, 1215; *Jour. of the American Med. Assn.*, 1899.

[22] *Compt. rend. Soc. de biol.*, 1893, xlv, 741; XI Internat. med. Cong. zu Roma; *Berliner klin. Woch.*, 1894, xxxi, 479.

[23] *Virchow's Arch.*, 1896, cxlv, 546.

[24] *Cent. für allg. Path.*, 1904, xv, 667.

[25] *Arbeiten a. d. path. Inst. z. Helsingfors*, 1902, 209.

[26] Contributions to the Science of Medicine Dedicated to Wm. H. Welch, *Reports of the Johns Hopkins Hosp.*, 1900, xi, 297.

[27] *Jour. of Path. and Bact.*, 1901, vii, 214.

this means I have succeeded in producing lesions of a character and intensity not obtained by simple administration of chloroform. This statement is illustrated by the following experiment.

A dog (Chlor. xvii) weighing 16,650 grm. has received on four successive days 8.3 c.c. of chloroform (0.5 c.c. per kilo); on the fifth day, 0.5 c.c. of a 24-hour bouillon culture of *Bacillus coli* has been injected into the jugular vein. The animal has received subsequently six doses of chloroform (0.5 c.c. per kilo) and has died on the twenty-third day of the experiment, 24 hours after a second injection of *Bacillus coli* (0.25 c.c.). There has been well marked jaundice. The liver is smooth; pale yellow tissue surrounds hemorrhagic spots occupying the centers of the lobules. *Bacillus coli* has not been recovered from the liver, bile, heart's blood or kidney.

Almost the entire hepatic parenchyma has undergone disintegration and is represented by a narrow zone of intact cells encircling each portal space (Fig. 6). These cells exhibit moderate infiltration with fat. In a few places the supporting framework of the lobule remains and endothelial cells persist, but elsewhere all hepatic architecture is lost and an almost structureless ground work is infiltrated with lymphoid cells, large mononuclear cells, polynuclear leucocytes, red blood corpuscles in great number and remains of liver cells. In an intermediate zone between this central tissue and the thin fringe of intact liver cells about the portal spaces, there is conspicuous hyaline necrosis of liver cells (Fig. 7) which, fragmented and scattered, have lost their nuclei and stain brightly with eosin. Proliferation of connective tissue is in progress and cells having the characters of fibroblasts are abundant in contact with the living tissue about the portal spaces. The presence of numerous newly formed tubules resembling bile ducts affords evidence of proliferation of parenchymatous elements (Figs. 6 and 7). These tubules, at times obviously continuous with surviving columns of cells, are pushing their way from the periphery to the center of the lobule. Occasionally, groups of such tubules occur near what has been the center of the lobule; in such places there is delicate connective tissue rich in cellular elements.

The lesion in this experiment has reproduced, with considerable accuracy, certain instances of acute yellow atrophy with beginning regeneration of parenchyma. Part of the tissue is necrotic and hemorrhagic, whereas a small part is intact and the site of fatty

change. Fibrous tissue has begun to encroach upon the zone of necrosis and active proliferation of bile ducts is in progress.

In order to test the effect of bacterial infection upon production of cirrhosis by chloroform, two animals have been given equal quantities of chloroform; and one has received bacteria injected into the jugular vein.

A dog (Chlor. xxii) weighing 10,550 grm. has received 10 c.c. of chloroform (1 c.c. per kilo) on three successive days; on the fourth day of the experiment it has lost 850 grams of body weight. On the 8th, 9th, 13th and 15th days it has received 10 c.c. of chloroform; on the 21st and 22d, 5 c.c. The animal has been killed on the 24th day. The liver is large (weighing 513 grm. = 1/21 of the original body weight) and pale yellow in color. There is no evidence of portal obstruction. Agar-agar inoculated from the liver, bile and kidneys remains sterile.

A dog (Chlor. xxiii) weighing 10,500 grm. has received chloroform in quantities and at intervals identical with those recorded for the preceding animal. On the fourth day of the experiment it has lost 500 grams of body weight and has received into the jugular vein 0.5 c.c. of a 24-hour bouillon culture of *Bacillus coli.* On the day following inoculation the animal has been very sick and for a time has appeared to be moribund. After the eleventh day of the experiment there has been jaundice. Death has occurred on the 24th day. The veins of mesentery and omentum are moderately distended, the pancreas is œdematous throughout and the peritoneal cavity contains 21 c.c. of serous fluid. *Bacillus coli* has been cultivated from the kidney but not from the liver or bile. The liver is small (309 grm. = 1/34 of original body weight) and pale yellow with lobulation sharply marked by yellow zones surrounding red spots.

In the liver of the animal (Dog Chlor. xxii) which has received chloroform alone, there is advanced, diffusely distributed fatty degeneration of the hepatic parenchyma (Fig. 9). Fibrous tissue in immediate contact with some of the central veins is slightly thickened and here are collected a few polynuclear leucocytes and small round cells, but changes affecting the interstitial tissue are trivial.

In the liver of the animal (Dog Chlor. xxiii) which has received both chloroform and *Bacillus coli,* on the contrary, masses and strands of cellular fibrous tissue subdivide the parenchyma into islands of which the relation to the original lobules is not always evident (Fig. 8). This new connective tissue forms at least half of the sectional area. About portal spaces fibrous tissue is not increased and, though central veins are often unrecognizable, contact with a portal space often shows that the central part of the

lobule is occupied by fibrous tissue containing a group of tortuous and enormously hypertrophied bile ducts obviously newly formed (Fig. 10). A circular zone of intact but fatty parenchyma often surrounds this central area. Small newly formed bile ducts radiate from this zone of parenchyma toward the central group of ducts. The smaller ducts are formed by low flat or cubical cells, but the larger more central ducts are formed by high columnar cells and have a wide lumen often filled with polynuclear leucocytes. The enormous multiplication of these duct-like structures is shown in the figures (Figs. 8 and 10).

A quantity of chloroform which alone has caused only fatty degeneration has in association with a single large dose of *Bacillus coli* produced advanced hepatic cirrhosis within a period of twenty-three days. A noteworthy feature of the lesion reproduced in subsequent experiments with Bacillus coli is the active proliferation and hypertrophy of bile ducts newly formed in situations far removed from the bile ducts of the portal spaces. Cirrhosis and multiplication of bile ducts is far more advanced at the end of twenty three days than in those experiments in which larger doses of chloroform unaccompanied by bacterial infection have been continued during two or three months.

In the following experiments, administration of chloroform in relatively small amounts has been accompanied by bacterial inoculation.

A dog (Chlor. xxi) weighing 7550 grm. has received 15.2 c.c. of chloroform (2 c.c. per kilogram of body weight) and on the following day 0.5 c.c. of a 24-hour bouillon culture of *Bacillus coli.* It has subsequently received twelve doses of 7.1 c.c. of chloroform, the total amount of chloroform administered being 104.5 c.c., and two injections of *Bacillus coli* (0.04 and 0.3 c.c.). There has been jaundice. Death has occurred at the end of thirty days. The liver is pale yellowish brown and weighs 260 grm. (1/29 of original body weight).

In sections for microscopical examination, the central part implicating approximately one-third of each lobule is replaced by loose cellular connective tissue containing many mononuclear cells. Where well-preserved columns of liver cells are in contact with this cellular tissue there are conspicuous tubules composed of cubical cells, arranged about a conspicuous lumen; these tubules, though

larger, have the characters of the bile ducts in the portal spaces but are separated from them by a wide zone of hepatic parenchyma. It is not improbable that this animal received its first injection of bacteria at a time when the liver was little injured, and subsequent small quantities of bacteria have had little effect upon the partially immunized animal.

In another experiment, a dog has received an immunizing dose of *Bacillus coli* and has subsequently received chloroform together with small quantities of *Bacillus coli* injected into the jugular vein.

A dog (Chlor. xxiv) weighing 5750 grm. has received 0.5 c.c. of a 24-hour bouillon culture of *Bacillus coli.* Sixteen days later, doses of chloroform (5 c.c.) have been given on three successive days; on the following day, the animal has received a very small quantity of Bacillus coli (0.01 c.c. of a bouillon culture). Later, chloroform has been administered interruptedly; in all 72.25 c.c. of chloroform have been given in 17 doses of 1 or 0.5 c.c. per kilogram of body weight during 43 days. Following the second injection of bacteria, small quantities of *Bacillus coli* (0.02, 0.02, 0.1, 0.05, 0.1, 0.1 and 0.2, 0.25 respectively at intervals of from three to ten days) have been injected into the jugular vein. Death, preceded by jaundice, has occurred 43 days after the first administration of chloroform. The liver is pale reddish yellow and relatively large (245 grm. = 1/24 of original body weight).

The parenchyma of the liver has undergone advanced degenerative change so that only a few cells in immediate contact with the portal spaces exhibit the usual staining characters of liver cells; even here the cells are finely vacuolated, but elsewhere all living cells are distended with large vacuoles, indicating advanced diffusely distributed infiltration with fat. Scattered among these fatty cells and more abundant near the central vein are numerous conspicuous groups of liver cells and remains of cells which have undergone hyaline necrosis. Such cells have lost their nuclei or contain particles of structureless nuclear substance. There is no newly-formed connective tissue, but particularly in the central part of the lobule there is infiltration with small and large mononuclear cells and with polynuclear leucocytes. A noteworthy feature of the organ is the presence of numerous newly formed bile ducts which are thickly scattered between the fatty liver cells and occur in the absence of new formation of interstitial tissue. In some instances these ducts are composed of cubical cells and have an inconspicuous lumen; in other instances, they are formed by high columnar cells ranged

about a conspicuous lumen. Such newly formed bile ducts are more numerous near the center than at the periphery of the lobule and no relationship to bile ducts within the portal spaces can be found.

In order to determine if the strain of colon bacillus which has been employed in the foregoing experiment is capable of producing hepatic lesions, large quantities have been injected at frequent intervals into the jugular vein of two animals.

A dog (B. C. 5) weighing 7,800 grm. has repeatedly received intravenously injections of *Bacillus coli* grown 24 hours in bouillon as follows: 1st day, 0.2 c.c.; 5th, 0.1; 8th, 0.1; 12th, 0.1; 15th, 0.25; 20th, 0.5; 24th, 0.25; 28th, 0.25; 31st, 0.5; 35th, 0.5; 41st, 0.5; 45th, 0.5; 48th, 0.5; 50th, 0.5; 54th, 0.5; 61st, 0.5. The animal has lost weight, weighing 5500 grm. on the 65th day of the experiment; on this day it has been killed. The liver is large, weighing 420 grm. (1/19 of original body weight), smooth and pale reddish brown in color. The gall bladder is small and its wall is thicker than usual; it contains clear yellow viscid bile in which are soft solid particles.

The hepatic cells are diffusely infiltrated with fat but there are no other noteworthy changes in the liver.

A dog (B. C. 7) weighing 10,950 grm. has received intravascular injections of bouillon cultures of *Bacillus coli* as follows: 1st day, 0.06 c.c.; 15th, 0.2; 19th, 0.1; 26th, 0.1; 29th, 0.25; 34th, 0.5; 38th, 0.5; 42d, 0.5; 45th, 0.5; 49th, 0.5; 55th, 0.5; 59th, 0.5; 62d, 0.5; 64th, 0.5; 68th, 0.5; 75th, 0.5. The animal has become thin and somewhat weak, weighing 8,500 grm. on the 79th day of the experiment; on this day it has been killed. The liver, weighing 394 grm. (1/28 of original body weight), is smooth, soft, brownish red and appears to be normal.

The liver shows infiltration with fat but is otherwise normal.

Further evidence that the repair of hepatic necrosis may be retarded by bacterial infection has been obtained by the employment of streptococcus. The microörganism employed has been obtained from the heart's blood and liver of a man dying with vegetative endocarditis of the aortic valve, associated with atrophic cirrhosis of the liver. It forms long chains and in bouillon tends to grow in small clumps which leave the medium clear.

In the following experiment two animals of approximately equal weight have received like quantities of chloroform administered in the same way to each animal. One of them has received repeated intravascular injections of *Streptococcus pyogenes;* a third animal has received *Streptococcus pyogenes* in the same quantity and at the same time.

A dog (Chlor. xxxii) weighing 8,350 grm. has received on three successive days 4 c.c. of chloroform (0.5 c.c. per kilogram of body weight); on the fourth day it had lost 450 grm. of body weight. It has received the same quantity of chloroform on the 7th, 8th, 12th, 17th and 18th day of the experiment, the total quantity administered being 32 c.c. Following the last dose of chloroform there has been jaundice which later has disappeared. The animal has been killed on the 28th day. There is a small amount of fluid (5 c.c.) in the peritoneal cavity and some œdema of the pancreas. The liver weighs 310 grm. (1/27 of original body weight) and is reddish yellow in color. No bacteria grew from liver or bile.

A dog (Chlor. xxxi) weighing 8,250 grm. has received chloroform in quantity and at intervals corresponding with those just described. Eight days before administration of chloroform, 0.08 c.c. of a 24-hour bouillon culture of *Bacillus coli* had been injected into the jugular vein. Immediately after the first dose of chloroform, 0.025 c.c. of a similar culture has been injected intravenously. On the 4th day after first administration of chloroform the animal has lost 200 grm. of body weight. There have been subsequent injections of streptococci on the following days (dating from first administration of chloroform): 7th, 0.05 c.c.; 13th, 0.2 c.c.; 19th, 0.2 c.c.; 21st, 0.3 c.c.; 24th, 0.3 c.c. The animal has died on the 28th day. There is advanced suppurative pericarditis, the pericardial cavity being distended with 500 c.c. of sero-purulent fluid. The liver weighs 238 grm. (1/35 of original body weight) and is deep red. Culture media inoculated from the liver and bile remain sterile.

A dog (Chlor. St. xxxiii) weighing 10,550 grm. has received streptococci in quantities corresponding with those administered on the 1st, 7th, 13th, 19th, 21st and 24th days to the foregoing animal. On the 28th day the animal has been killed. The liver weighing 410 grm. (1/27 of the original body weight) is normal in appearance. Culture media inoculated from liver and bile remain sterile.

In the animal which received chloroform alone (Chlor. xxxii) the liver cells exhibit advanced diffusely distributed fatty degeneration (Fig. 12). Within part of the lobules there is no other change, but in the center of others about the central vein is loose meshed connective tissue, containing widely dilated capillaries and a small number of large and small mononuclear cells. In the margin of this tissue in many places occur newly formed bile ducts.

In the animal which received both chloroform and streptococcus (Chlor. xxxi) changes in the liver are considerably more extensive (Fig. 11). About each central vein is an area implicating about one-third of the space from central vein to portal space in which liver cells are replaced by a loose vascular tissue containing numerous mononuclear cells. In immediate contact with the vein these cells are small and very numerous, whereas at a greater distance

they are larger and contain abundant pigment. Newly formed bile ducts in moderate number occur within and at the margin of the tissue. The liver cells which are well preserved and exhibit little infiltration with fat are often arranged in tubular columns with nuclei near either margin of the columns.

In the animal (Chlor. xxxiii) which for control received repeated intravascular injections of streptococcus, the liver cells exhibit infiltration with fat in moderate quantity. There is no destruction of parenchyma or other noteworthy alteration. In other animals (Chlor. xxviii and xxxvii) which have been repeatedly inoculated with the same strain of *Streptococcus pyogenes,* no noteworthy hepatic lesion has been produced.

In the following experiment, two animals have received a single large dose of chloroform; one has been allowed to recover whereas the other has received repeated intravenous injections of *Streptococcus pyogenes.*

A dog (Chlor. xxxix) weighing 4,850 grm. has received by mouth 15 c.c. of chloroform. On the following day it has appeared sick but has quickly recovered, losing at the end of two days 100 grm. of body weight. On the 15th day of the experiment the animal has been killed. The liver is not enlarged; its surface is smooth and its color and texture are those of the normal organ.

A dog (Chlor. xxxviii) weighing 6,000 grm. has received by mouth 15 c.c. of chloroform; almost immediately 0.5 c.c. of a bouillon culture of *Streptococcus pyogenes* has been injected into the jugular vein. During the next two days the animal has been very sick and has lost 450 grm. of body weight. On the 4th, 6th, 8th and 12th day of the experiment it has received 0.25 c.c. of a 24-hour bouillon culture of streptococcus; it has died on the 15th day. The liver is much enlarged with homogeneous deep red color and turgid appearance. In places upon the surface is a thin layer of fibrin. Lobulation is not definable on the deep red surface.

The liver of the animal (Chlor. xxxix) which has received two weeks before its death a single large dose of chloroform exhibits little change (Fig. 14). The liver cells contain fine droplets of fat but are otherwise normal; there is no increase of interstitial tissue.

In the liver of the animal (Chlor. xxxviii) which has received the same quantity of chloroform followed by repeated intravascular inoculation with streptococci (Fig. 13), a conspicuous mass of loose meshed connective tissue replaces the hepatic parenchyma about each central vein and at times extending from one to another central vein,

maps out islands of parenchyma, each with a portal space in its center. The new interstitial tissue is rich in cells and contains abundant blood, some of which has apparently escaped from the vessels. The tissue contains many cells resembling fibroblasts but new formation of intercellular substance is not evident. In immediate contact with portal spaces and in the adjacent parenchyma are widely dilated blood vessels at times collected into groups resembling angiomata. Such dilated vessels, which are apparently preëxisting capillaries which have undergone immense dilatation, do not occur in the central part of the lobule. It is possible that this localized dilatation of capillaries is referable to an increased pressure in the domain of the hepatic artery, dilating the capillaries at the junction of arterial with portal vascular systems, but direct evidence in favor of this suggestion has not been obtained.

The experiment shows that infection with streptococcus retards the regeneration of hepatic parenchyma after destruction caused by chloroform.

The experiments in which chloroform has been administered interruptedly during a considerable period show that chloroform is capable of causing cirrhosis so advanced that the portal circulation is obstructed. Dilatation of superficial abdomen veins is recognizable during life; venous engorgement in the portal domain, œdema of the pancreas and moderate accumulation of ascitic fluid have accompanied the hepatic lesion. The relation of necrosis of the liver to new growth of interstitial tissue is obvious in these experiments for both occur primarily in contact with the central vein of the lobule. Proliferation of bile ducts occurs and newly formed tubules may appear near the center of the lobule, often separated from the ducts of the portal space by a broad zone of relatively normal parenchyma.

Bacterial infection in association with intoxication by hepatic poisons such as chloroform and phosphorus have reproduced the peculiar mid-zonal necrosis of the liver which in man is almost invariably found in association with acute bacterial infection. Neither chloroform nor phosphorus alone produces this lesion but either when associated with the bacteria which have been employed may produce it.

The same combination of bacteria and poison, doubtless acting with greater intensity, reproduces a lesion which closely resembles acute yellow atrophy in man. There is necrosis and hemorrhage; accumulation of wandering cells and formation of connective tissue replacing parenchymatous elements quickly follows. The regenerative changes both of parenchyma and of interstitial tissue observable in man occur in the experimental lesion. New formation of bile ducts is a conspicuous feature of these regenerative changes.

The experiments have shown that bacteria in association with toxic substances such as chloroform and phosphorus having a special affinity for the liver may produce changes which neither the poison nor the bacteria can cause when acting alone. The possibility that disturbances of metabolism in man may produce changes in the liver similar to those caused by such poisons is well illustrated by the toxemia of pregnancy in which persistent vomiting is associated with central necrosis of the hepatic lobule similar to that caused by chloroform. The experiments show that the activity of an hepatic poison may be so intensified by bacterial infection that a quantity of the poison which alone produces little change may, in combination with the bacteria (*Bacillus coli* and *Streptococcus pyogenes* have been employed), cause destruction of almost the entire hepatic parenchyma. Bacteria, *e. g.*, colon bacillus, which have little pathogenicity for the normal animal, are virulent for the animal whose liver has been injured by chloroform or phosphorus. It is probable that those instances of acute yellow atrophy which accompany infection with *Streptococcus pyogenes* are dependent upon some disturbance of metabolism or other form of intoxication which has rendered the liver unusually susceptible.

The experiments furthermore show that bacterial infection can influence and even determine the development of cirrhosis of the liver. Degenerative changes which usually undergo rapid repair may cause sclerosis when combined with bacterial infection. Whereas cirrhosis may be caused by chloroform without bacterial inoculation, introduction of bacteria hastens the progress of the chronic change. The poison in large quantity rapidly causes death, but a much smaller quantity in association with a relatively non-pathogenic microörganism produces a lesion with which recovery

is possible. Such insults repeated at intervals produce the chronic changes of cirrhosis. By administration of chloroform, accompanied by intravenous inoculation with *Bacillus coli,* it has been possible to produce advanced hepatic cirrhosis accompanied by active new formation of bile ducts within a period of twenty-three days.

EXPLANATION OF PLATES.

PLATE XXXI.

FIG. 1. Showing cirrhosis of the liver with new formation of bile ducts caused by administration of chloroform during two months (Dog. Chlor. i).

PLATE XXXII.

FIG. 2. Showing mid-zonal necrosis of the liver in a rabbit (Rabbit Phos. 5) which has received both phosphorus and streptococci.

FIG. 3. Showing the relatively normal liver of an animal (Rabbit Phos. 2) which has received phosphorus alone (in quantity corresponding with that administered to Rabbits Phos. 5 and 6). For comparison with Figs. 2 and 4.

PLATE XXXIII.

FIG. 4. Showing fatty degeneration and necrosis of the liver in a rabbit (Rabbit Phos. 6) which has received both phosphorus and streptococci.

FIG. 5. Showing the relatively normal liver of an animal (Rabbit Phos. 3) which has received phosphorus alone (in quantity corresponding with that administered to Rabbits Phos. 5 and 6). For comparison with Figs. 2 and 4.

PLATE XXXIV.

FIG. 6. Photograph of the liver of a dog (Chlor. xvii), showing a lesion resembling acute yellow atrophy of the liver; there is necrosis of parenchyma and beginning new formation of bile ducts.

FIG. 7. Drawing of the liver from the same animal (Chlor. xvii) with higher magnification showing isolated bile ducts in the loose connective tissue which has partially replaced the necrotic parenchyma in the central part of the lobule. There is a narrow zone of intact parenchyma in contact with the portal spaces. In an intermediate zone there is hyaline necrosis.

PLATE XXXV.

FIG. 8. Photograph of the liver of a dog (Chlor. xxiii) which has received chloroform plus *Bacillus coli* injected intravenously. There is advanced cirrhosis with new formation of bile ducts twenty-three days after the beginning of the experiment.

FIG. 9. Photograph of the liver of a dog (Chlor. xxii) which has received chloroform alone, administered in quantity equal to that received by Dog Chlor. xxiii. There is diffuse fatty degeneration. For comparison with Fig. 8.

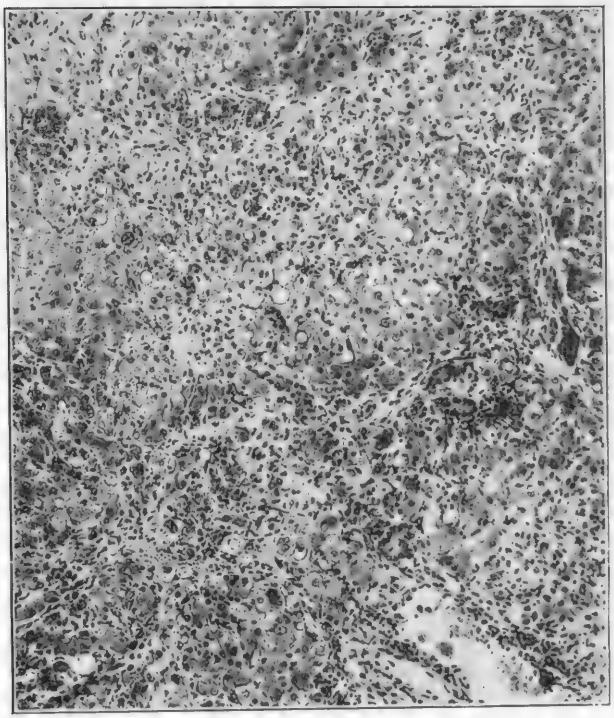

FIG. 1.

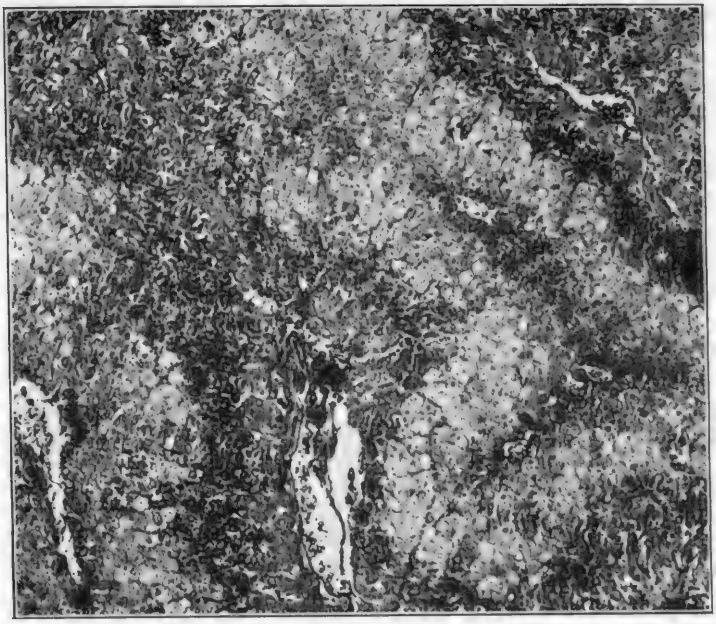

FIG. 2.

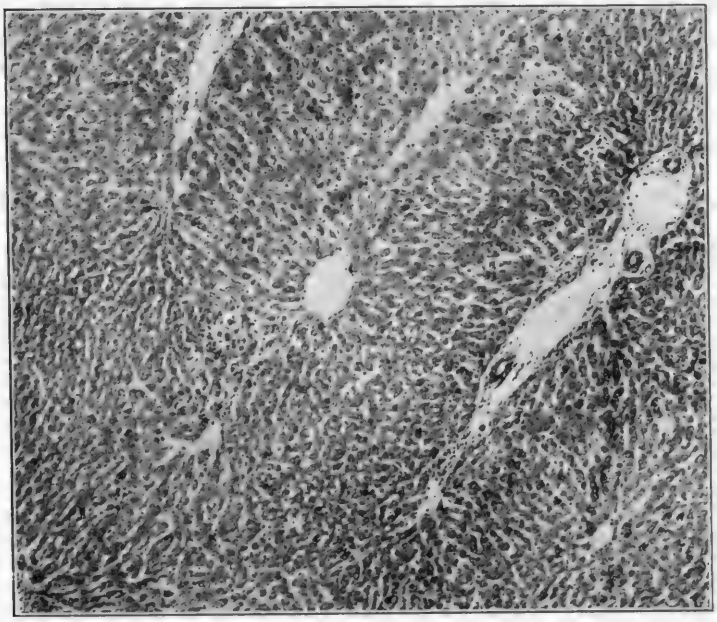

FIG. 3.

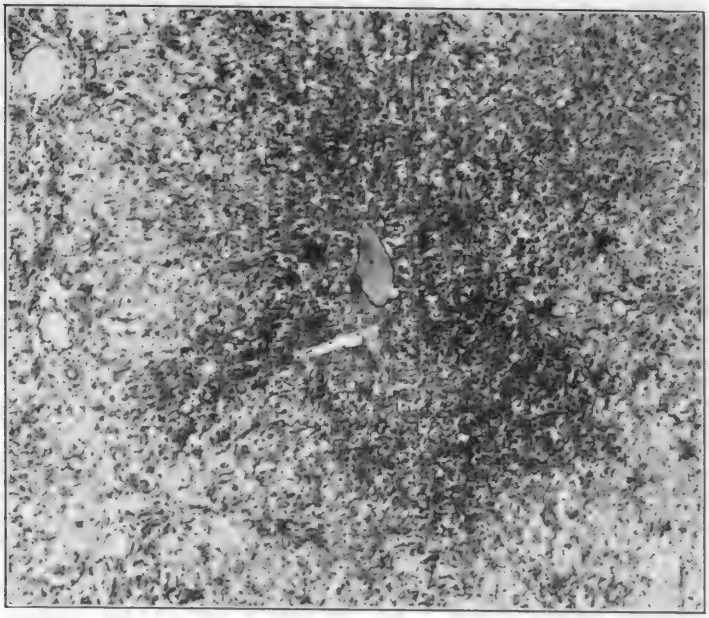

FIG. 4.

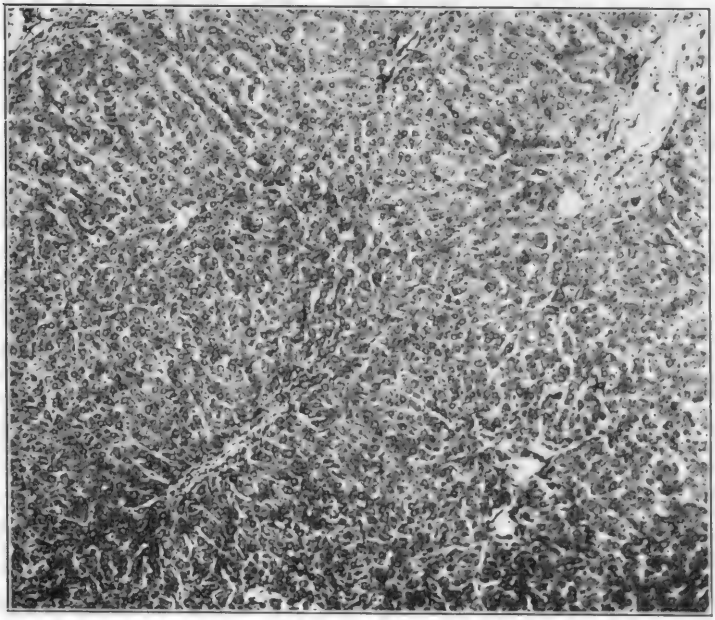

FIG. 5.

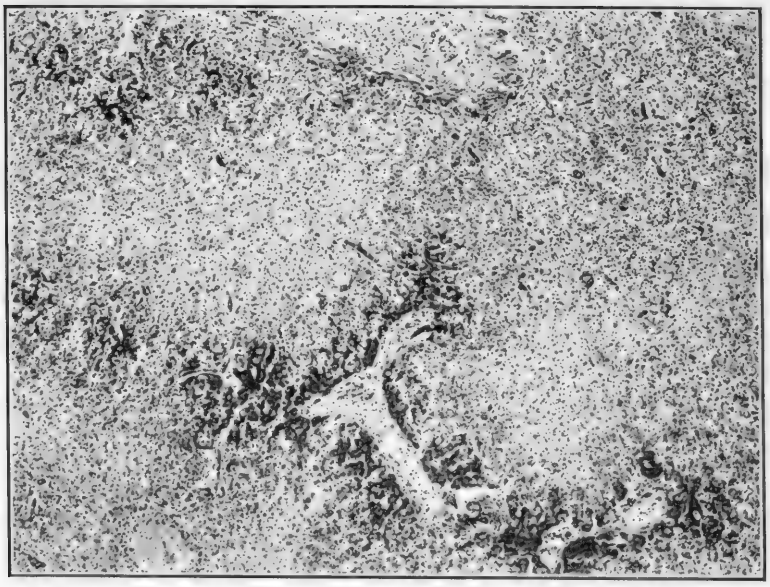

FIG. 6.

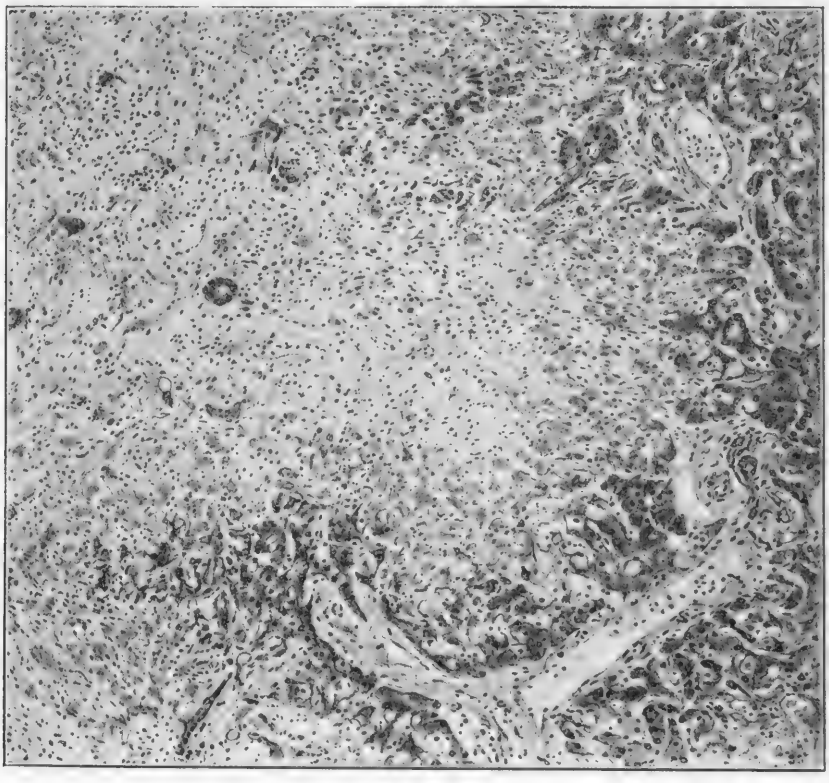

FIG. 7.

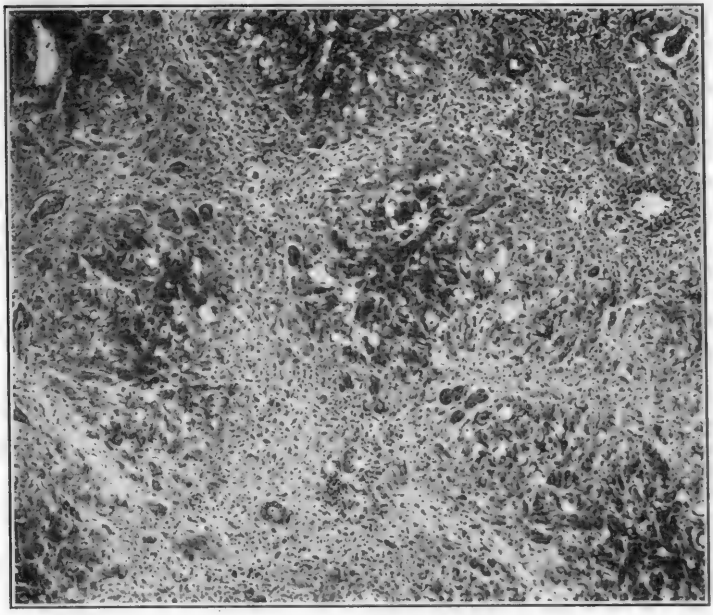

FIG. 8.

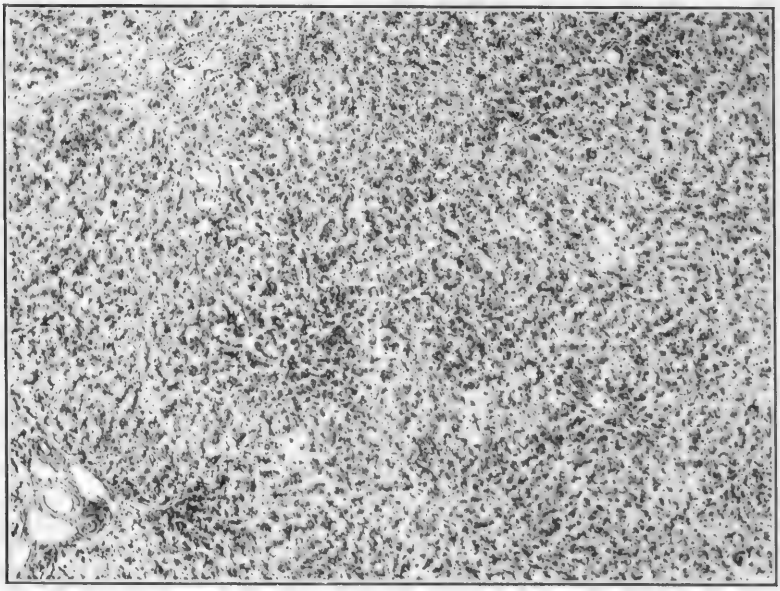

FIG. 9.

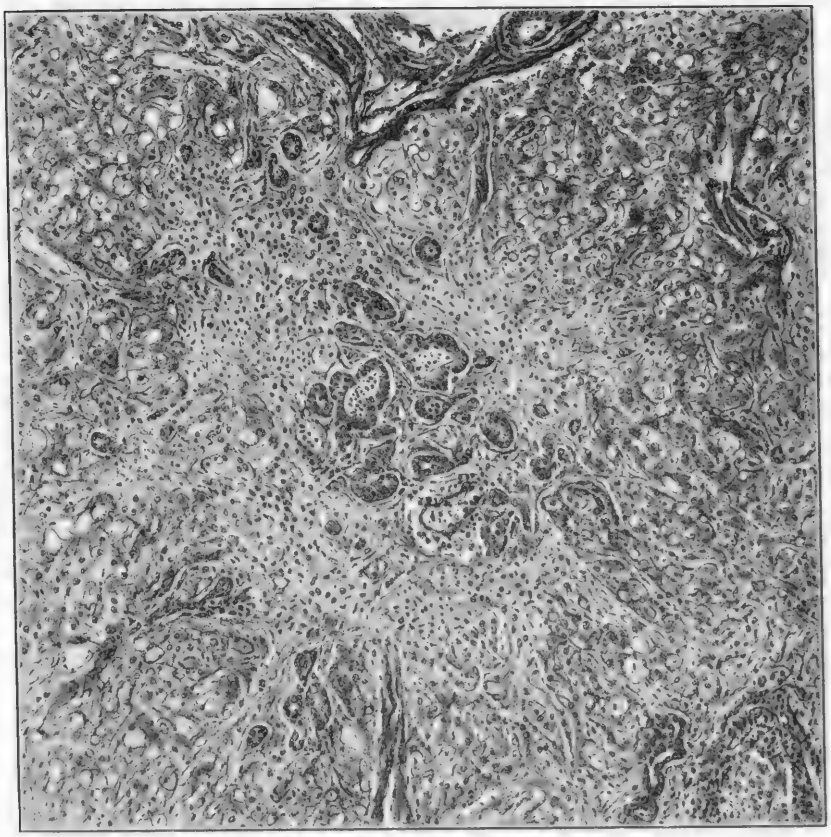

FIG. 10.

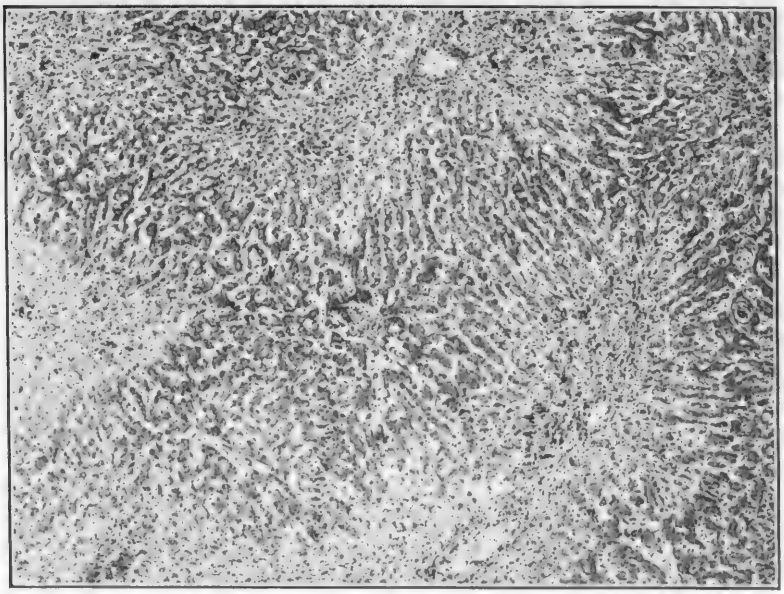

FIG. 11.

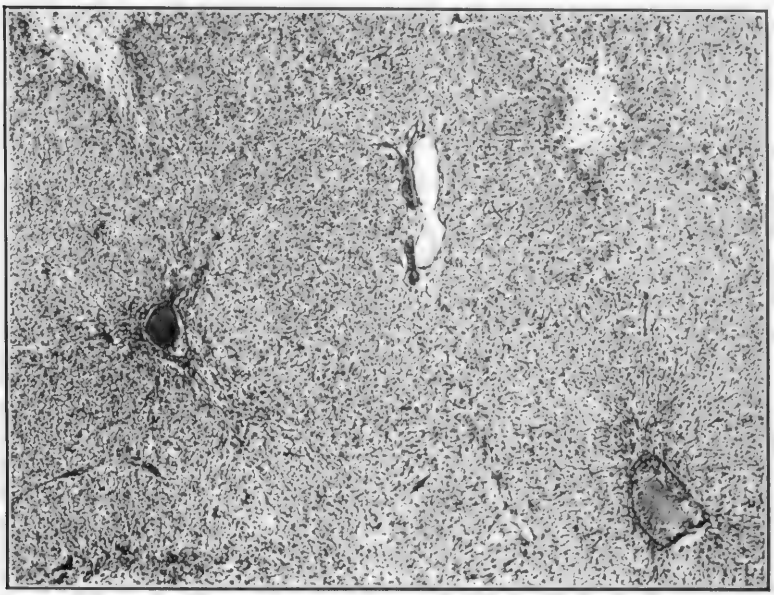

FIG. 12.

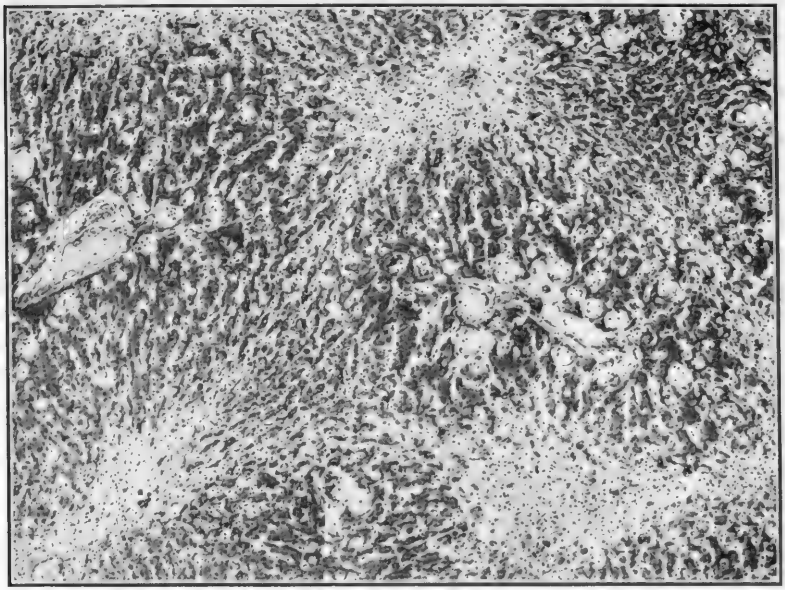

FIG. 13.

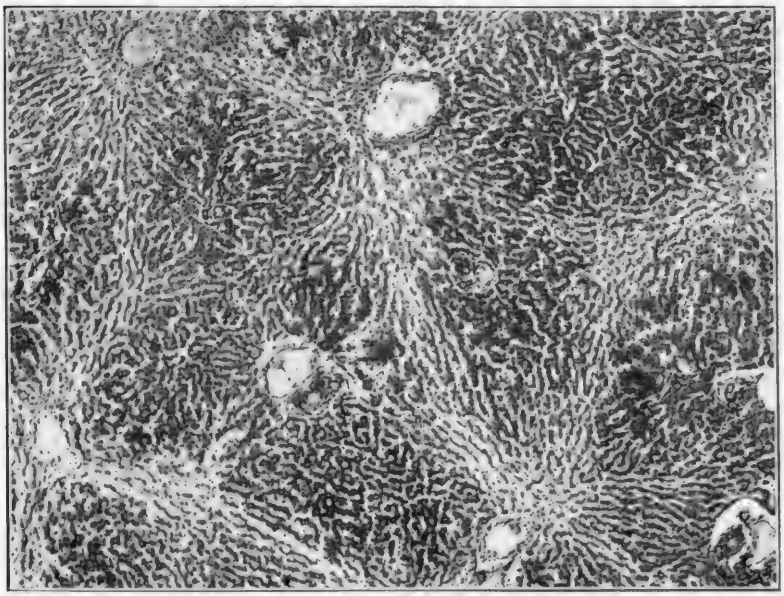

FIG. 14.

PLATE XXXVI.

FIG. 10. Drawing of the liver (Chlor. xxiii) shown in Figure 8. Newly formed fibrous tissue most abundant about the central vein of the lobule (see center of figure) replaces and divides the parenchyma. There is enormous proliferation of bile ducts and polynuclear leucocytes are abundant within the larger of the new ducts which occupy the central part of the lobule.

PLATE XXXVII.

FIG. 11. Photograph showing the liver of a dog (Chlor. xxxi) which has received chloroform and streptococci. The central part of each lobule is replaced by interstitial tissue.

FIG. 12. Photograph showing the liver of a dog (Chlor. xxxii) which has received chloroform in quantity equal to that received by Dog Chlor. xxxi. There is diffuse fatty degeneration and some increase of interstitial tissue. For comparison with Fig. 11.

PLATE XXXVIII.

FIG. 13. Photograph showing the liver of a dog (Chlor. xxxviii) which has received a single large dose of chloroform followed by intravenous inoculation with streptococci. Interstitial tissue replaces the parenchyma in the center of each lobule; there is enormous dilatation of capillaries about the portal spaces.

FIG. 14. Photograph showing the liver of a dog (Chlor. xxxix) which has received a single large dose of chloroform (corresponding with that administered to Dog. Chlor xxxviii). The liver shows no noteworthy change. For comparison with the foregoing figure.

STUDIES ON WATER DRINKING.[1]

II. The Metabolic Influence of Copious Water Drinking With Meals.

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(*From the Laboratory of Physiological Chemistry of the University of Illinois.*)

INTRODUCTION.

Although there have been many investigations made upon the influence of copious water drinking,[2] yet nowhere, in so far as we have been able to discover from a careful study of the literature, has there been made a carefully conducted experiment or series of experiments upon human subjects for the purpose of obtaining data as to the effect of the copious ingestion of water *with meals.* The reason why our literature contains records of no such experiment is not far to seek. The medical profession almost unanimously advise strongly against the drinking of large amounts of water at meal time. The desirable features following the liberal use of water taken at the proper time are thoroughly appreciated, but any suggestion as to the taking of water in large quantity with meals is strongly antagonized. Such being the attitude of the medical profession, it has therefore been a natural consequence that those interested in the study of medical problems should fail to attempt to place the theory upon an experimental basis. Why investigate something which is self evident! At first thought, the present day theory as to the inadvisability of ingesting large volumes of water with meals sounds extremely reasonable. The principal objection to the copious ingestion of the fluid is based upon the supposition that the excess water dilutes the gastric juice and thus disturbs to

[1] Reported before the section on Pathology and Physiology of the American Medical Association, Atlantic City, June, 1909. Received for publication January 20, 1910.

[2] For literature and the first article in the series see Hawk, *Univ. of Pennsylvania Med. Bull.*, 1905, xviii, 7.

a marked degree the normal rhythm of the digestive functions. However we can no longer entertain the old belief that such is the case. The experiments recently reported by Foster and Lambert[3] indicate most convincingly that the entrance of water into the stomach does not produce a dilute gastric juice of lowered acidity but rather that the entrance of this fluid acts as a distinct stimulation to the gastric secretion and that the juice, although secreted in larger volume than previous to the entrance of the water, nevertheless shows a higher concentration of acid than does that juice which is secreted under ordinary conditions.

In the experiment we are about to report the subject was placed on a normal, constant diet, and by means of a preliminary period of sufficient length, was brought to a condition of approximate nitrogen equilibrium. At that point, 1,000 cubic centimeters of water was added to each meal and continued thus through a period of five days. Immediately following this period came a final period of eight days during which the original normal constant diet was again maintained and the after effects of the copious water ingestion observed.

The urine was collected in twenty-four-hour samples while the feces was collected in period samples. The foods with the exception of milk, were analyzed before the experiment began, after first preparing a satisfactory sample of each of the foods to be used. The milk was analyzed at frequent intervals during the experiment. Thus accurate knowledge of the income and outgo of nitrogen was obtained and reliable conclusions made possible.

DESCRIPTION OF EXPERIMENT.

Subject, Daily Schedule and Diet.—The subject of this experiment was a young man twenty-two years of age, weighing at the commencement of the experiment 71.69 kilograms.

The daily schedule for the preliminary and final periods of the experiment was as follows:

6:30 a. m.	Arose, defecated, weighed.
7:30 a. m.	Breakfasted (100 cubic centimeters of water with meal).
10:00 a. m.	200 cubic centimeters of water taken.
12:30 p. m.	Noon meal (100 cubic centimeters of water with meal).
3:30 p. m.	200 cubic centimeters of water taken.
6:15 p. m.	Dinner (100 cubic centimeters of water with meal).
8:30 p. m.	200 cubic centimeters of water taken.

[3] Foster and Lambert, *Jour. of Exper. Med.*, 1908, x, 820–840.

On the days of the increased water ingestion, 1000 cubic centimeters of water was added to each of the three meals. This additional water was to be drunk throughout the progress of the meal, care being taken to masticate the food properly.

The articles of diet were butter, peanut-butter, whole milk, soda crackers, corn-flakes and sugar, 25 grams of butter, 20 grams of peanut-butter, 400 grams of whole milk, 100 grams of soda crackers, 25 grams of corn-flakes, and 12 grams of sugar constituting the menu for one meal. Each meal of the day was absolutely identical with the others, and thus was procured an absolute uniformity of diet throughout the entire experiment. The daily diet contained 14.654 grams of nitrogen.

The water used in the experiment was from the regular University supply. This water analyzed as follows:[4]

ANALYSIS OF WATER FROM UNIVERSITY OF ILLINOIS WATER SUPPLY.

Ions.		Parts per million.	Hypothetical combinations.		Parts per million.	Grains per U. S. gallon.
Potassium,	K	2.6	Potassium nitrate,	KNO_3	1.1	0.06
Sodium,	Na	29.0	Potassium chloride,	KCl	2.9	0.17
Ammonium,	NH_4	2.3	Sodium chloride,	NaCl	3.5	0.20
Magnesium,	Mg	34.9	Sodium sulphate,	Na_2SO_4	3.6	0.21
Calcium,	Ca	70.1	Sodium carbonate,	Na_2CO_3	60.5	3.52
Iron,	Fe	1.0	Ammonium carbonate,	$(NH_4)_2CO_3$	6.1	0.36
Aluminium,	Al	1.3	Magnesium carbonate,	$MgCO_3$	121.2	7.07
Nitrate,	NO_3	.7	Calcium carbonate,	$CaCO_3$	175.2	10.22
Chlorine,	Cl	3.5	Iron carbonate,	$FeCO_3$	2.1	0.12
Sulphate,	SO_4	2.3	Alumina,	Al_2O_3	2.5	0.15
Silica,	SiO_2	18.9	Silica,	SiO_2	18.9	1.10
			Total,		397.6	23.18

Before using this water it was softened by adding to 30 liters, 5 liters of saturated lime water.[5] After settling, the water was filtered and used. The alkalinity of this water was 70 to phenolphthalein and 180 to methyl orange. Its hardness determined by soap solution was 92 parts per million.

Collection of Urine and Feces.—The experimental day extended from 7 a. m. to 7 p. m. If possible, the subject defecated each morning before breakfast and then immediately determined his body weight, without clothes. This schedule was followed carefully every day throughout the experiment. It is, therefore, obvious that each day was begun under uniform conditions, so far as it was possible to control them.

The feces was collected in period samples. To secure satisfactory differentiation of the feces of one period from the feces of the following period, a gelatin capsule containing about 0.2 gram of powdered charcoal was taken just before

[4] Bartow and Lindgren, Chemical and Biological Survey of the Waters of Illinois, *Univ. of Illinois Bulletin*, 1908, vi, 33.

[5] For the preparation and testing of this water we are indebted to Mr. L. I. Birdsall of the Illinois State Water Survey.

breakfast of the first day of the new period. This powdered charcoal formed a more or less regular black mass in the fecal matter and thus rendered the separation of the feces of the one period from that of the succeeding period a comparatively simple matter.

Methods of Analysis.[6]—In the urinary analysis the following methods were used:

1. Total nitrogen: Kjeldahl method.
2. Urea: Benedict and Gephart method.
3. Ammonia: Folin method.
4. Creatinine: Folin method.
5. Creatine: Folin-Benedict method.

In the quantitative determination of the fecal bacteria, the method of Strasburger,[7] as modified by Dr. Ward J. MacNeal of this University, was employed.[8]

DISCUSSION OF RESULTS.

Excretion of Nitrogen—The nitrogen content of the urine for the fifth day of the preliminary period was 13.084 grams, and for the sixth day, 13.183 grams. The elimination of nitrogen by the urine being so very uniform for these two days, signifying that a satisfactory plane had been reached and that nitrogen equilibrium was fairly well established, 3,000 cubic centimeters of water were added to the diet, on the following day, the seventh of the experiment, 1,000 cubic centimeters being taken during the progress of each meal. The effect of this large addition to the amount of water taken was seen immediately not only in the very low specific gravity, of the increased volume of urine, but also in the marked increase in the nitrogen excretion for the day. This large amount of water was taken, as we have seen, when the body was in a condition of approximate nitrogen equilibrium and excreting daily an average of 13.134 grams of nitrogen by the urine. Table I shows that this average excretion of nitrogen for the preliminary period was greatly increased by the drinking of 3,000 additional cubic centimeters of water, and that the first day of the water period showed an excretion of 14.161 grams of nitrogen by the urine, an increase of 1.027 grams or 7.82 per cent. (Table III), above the average elimination of the preliminary period. The excretion of nitrogen by the urine on the second day of the water period, while not so great as that of

[6] Hawk, Practical Physiological Chemistry, 2d edition, Philadelphia, 1909, pp. 379–393.

[7] Strasburger, *Zeit. f. klin. Med.*, 1902, xlvi, 413.

[8] MacNeal, Latzer and Kerr, *Jour. of Infect. Dis.*, 1909, vi, 123.

the first day, was still in excess of the average normal output of the preliminary period. Here, with the addition of 3,000 cubic centimeters of water to the constant diet at meal times, we obtained an excretion of 13.491 grams of nitrogen, this being 0.357 gram above the average daily excretion for the preliminary period. On the next two days of the water period, the nitrogen excretion by the urine fell slightly below normal being 12,981 grams for the ninth day and 12.976 grams for the tenth day. On the eleventh day of the experiment, however, the final day of the water period, the excretion was found to be 13.138 grams, which is approximately the same as that of the last two days of the preliminary period. Hence we see, by referring to the data, that a daily addition of 3,000 cubic centimeters of water, to a constant diet, caused a total increase of 1.077 gram of nitrogen for the period (Table IV).

On the twelfth day of the experiment, the final period of the investigation was begun. The diet of this day, as well as that of the seven succeeding days was the same constant diet as that fed during the preliminary period. This diet on the first day of the new normal period gave an excretion of 11.823 grams of nitrogen as compared with the average daily excretion of 13.134 grams of nitrogen of the preliminary period. This showed a decided decrease in the excretion of nitrogen, being 1.311 gram less than the normal excretion of the preliminary period and 1.526 gram lower than the average excretion for the water period. On the thirteenth day there was a slight increase of 0.013 gram in the excretion, but on the fourteenth there occurred a rise of over one gram. On the last three days of the final period the excretion had practically returned to normal again, the seventeenth day showing an excretion of 13.030 grams, while the excretions of the eigthteenth and nineteenth days were respectively 12.905 grams and 13.160 grams, the average of these three days giving us a value of 13.032 grams as compared to the average output of 13.134 grams per day for the last two days of the preliminary period. This indicates very clearly that the original condition of nitrogen elimination by the urine which existed before the ingestion of the large volume of water had been reëstablished. Therefore, it is evident that the effect upon the excretion of nitrogen by the urine, of the addition of fifteen liters

of water to the diet during meal time may be measured by taking into consideration all the daily excretions of nitrogen from the first day of the water period until the original conditions were re-established upon the seventeenth day of the experiment. By referring to Tables I and IV we see that the total increase in the excretion of nitrogen during the water period was 1.077 grams, all occurring on the first day of increased water ingestion. Also we may see that in the final period before the subject returned to equilibrium again, there was a total decrease in the excretion of nitrogen amounting to 5.123 grams of nitrogen. Now subtracting 1.077 grams from 5.123 grams we find that there was a gain of 4.046 grams of nitrogen, judging from the urinary data alone. (See later discussions of income and outgo of nitrogen, p. 400.)

Excretion of Urea.—In general, the variations in the urea content of the urine followed very closely the variations in the total nitrogen. It is interesting to note, however, that the percentage of total nitrogen which appeared as urea (Table II) decreased from 88.95 per cent. on the first day of the water period to 85.86 per cent. on the second day of the water period, from which day to the end of the period there was an increasing percentage of the total nitrogen excreted as urea.

These decreased excretions of urea-nitrogen mentioned above were accompanied by increased excretions of ammonia-nitrogen. We believe the decrease in the urea output during these first days of the water period and the accompanying increase in the ammonia output to be due directly to the fact that the water stimulated the flow of gastric juice. The reasons for this belief are fully discussed in the following paragraph.

Excretion of Ammonia.—The average excretion of ammonia-nitrogen for the last two days of the preliminary period was 0.337 gram, or 2.56 per cent. of the total nitrogen excretion. On the first day of the water period the ammonia content of the urine increased (Table III) and remained higher for the whole water period. The average daily increase in ammonia-nitrogen for the water period was 0.159 gram, or an increase above the ammonia-nitrogen of the preliminary period of 47.18 per cent. (Table IV). This increased output of ammonia nitrogen during the water period

above that of the preliminary period seems to us capable of but one interpretation. It has been demonstrated that increased water ingestion stimulates the flow of gastric juice and that the increased volume of juice also possesses a higher concentration of acid.[9] It is apparent, therefore, that the hydrochloric acid above that used for digestive purposes is absorbed, forms ammonium chloride with a certain portion of the ammonia resulting from the protein catabolism, and is excreted in this form in the urine. The ammonia content of the urine is consequently raised and there is an accompanying decrease in the output of urea-nitrogen, since the ammonia used in neutralizing the excess hydrochloric acid would in the ordinary digestive cycle appear in the urine as urea. Proof that excess hydrochloric acid may thus increase the ammonia output has been furnished by Walter.[10] This investigator administered the acid to dogs and observed that about three-fourths of it was neutralized by the ammonia in the body whereas the major portion of the remaining one-fourth contributed toward the acidity of the urine.

Excretion of Creatinine.—The fact that the creatinine excretion was uniformly lower during the period of increased water ingestion than it was during the preliminary period rather substantiates the opinion that the increased nitrogen excretion accompanying copious water ingestion is due, at least in part, to increased protein catabolism.[11] At any rate we may thus interpret this observation, if we accept the view of Lefmann[12] that increased protein catabolism produces a decrease in the creatinine excretion. Our data also furnish definite verification of the finding of Lefmann, and of Levene and Kristeller[13] to the effect that decreased creatinine elimination is accompanied by an increased creatine output. An examination of Table I, will demonstrate this statement. No creatine was present in the urine until the low creatinine output of the water period was observed. This subject is more fully discussed on a later page.

[9] Foster and Lambert, *loc. cit.*
[10] Walter, *Arch. f. exper. Path.*, 1877, vii, 148.
[11] Hawk, *loc. cit.*
[12] Lefmann, *Zeit. f. physiol. Chem.*, 1908, lvii, 476.
[13] Levene and Kristeller, *American Jour. of Physiol.*, 1909, xxiv, 45.

In this connection data regarding the "creatinine coefficient"[14] are of interest. This coefficient is the *milligrams of creatinine-nitrogen per kilogram of body weight.* As we have just seen, the creatinine excretion was decreased through the influence of the copious intake of water. This fact would tend to lower the coefficient. At the same time also, the body weight was increasing, thus working toward a further lowering of the coefficient. Calculating this value for the three periods, we find that the coefficient of 8.89 obtained during the preliminary period was reduced to 8.36 during the water period (Table VII). The entire final period yields a coefficient of 8.70 whereas the final day of the experiment gives us a value of 8.88 which is practically the same as the coefficient of the preliminary period. The lower coefficient (8.70) obtained when the data of the whole period are taken into account is due to the fact that the low daily creatinine output instituted in the water period was continued during the first portion of the final period. Notwithstanding the lower coefficient of the water period, the muscular efficiency of the subject was apparently not lessened.

Excretion of Creatine.—Simultaneously with the ingestion of the large quantities of water, creatine appeared in the urine. The amount of creatine excreted varied considerably on the different days, but it continued to appear on each day of copious water drinking as well as on each of the first three days of the final period. The daily excretion of creatine-nitrogen for the water period ranged from 0.024 to 0.128 gram, the maximum output being registered during the final day of the increased water ingestion. The average daily excretion of creatine-nitrogen for the entire water period was 0.074 gram (Table IV), or 0.56 per cent. of the total nitrogen output. On the fourth day of the final period during which the 3000 cubic centimeters of water was eliminated from the diet, the creatine failed to appear and no trace of it could be found in the urine during the rest of the experiment.

The appearance of creatine in the urine under the influence of copious water drinking is a most significant feature, and apparently affords us a clue as to the actual source of at least a portion of the increase in the urinary nitrogen which accompanies the ingestion

[14] Shaffer, *American Jour. of Physiol.*, 1908, xxiii, 1.

of excessive quantities of water. As is well known, there are two hypotheses as to the source of this increase in the nitrogen output.[15] One view asserts that the increase is due simply to a flushing-out process, thus removing the nitrogenous end-products of previously disintegrated tissues. The other view is to the effect that this increased output of nitrogen results entirely from a stimulation of protein catabolism. According to this view, the nitrogen excess, instead of being composed of nitrogenous constituents which have resulted from some previous tissue cleavage and have not yet been excreted because the volume of fluid ingested was insufficient to afford a thorough flushing of the tissues, is due to protein catabolism promulgated through the agency of the increased water ingestion.

In all the investigations upon the influence of water drinking, made up to this time, there have never been obtained any experimental data, so far as we are aware, which place either of these hypotheses upon a firm foundation. However, in the present investigation our creatine data give us an indication as to the origin of at least a portion of the excess nitrogen excreted under these conditions. No urine of the preliminary period contained creatine, but as soon as the three liters of water was daily added to the diet, creatine at once appeared. It is probable that the creatine appeared because of the partial or complete disintegration of muscular tissue. We believe that the muscular tissue yielding the creatine could not by any chance have been disintegrated previous to the entrance of the large amount of water into the organism. We base our belief upon the fact that creatine is not a constituent of normal urine. Therefore, it appears to us entirely outside the realm of possibility that each of the nitrogenous end-products of muscle disintegration should be excreted under normal conditions, *except the creatine,* and that the latter should remain behind and make its appearance only when an unusual flushing occurs. The creatine was, therefore, at least in part, derived from muscular tissue which was wholly or in part disintegrated under the influence of the ingestion of the excessive amounts of water.

The observations of Lefmann, as before mentioned, are of inter-

[15] Hawk, *loc. cit.*

est in connection with our creatine and creatinine data. This observer concludes that increased protein catabolism produces, first an increase, then a decrease in the creatinine excretion. He further states that when the decreased creatinine output appears that there is an accompanying increase in the creatine output. Reference to Table I shows this latter point very clearly in the case of our water-drinking experiment. On the second day of the water period, the creatinine output was 0.616 gram and the creatine output, 0.024 gram. On the following day, however, under the influence of the added water the creatinine excretion fell to 0.589 gram and coincidentally the creatine excretion increased to 0.102 gram. These conditions regarding the output of these two substances were almost exactly duplicated on the two following days, *i. e.,* we observe a creatinine output of 0.608 gram accompanied by a creatine output of 0.055 gram, which are followed on the succeeding day by a decrease in creatinine to 0.589 gram and an accompanying increase in creatine, the output being the maximum creatine excretion of the experiment. These findings correspond with Lefmann's observations. We did not observe any increase in the creatinine excretion at the outset of the period of increased protein catabolism, as he suggests. On the contrary, the creatinine output was low throughout the water period and the normal rate of output for this urinary constituent was not assumed again until the fourth day of the final period.

Origin of Excreted Creatine.—If we consider the creatine output as representing disintegration of muscular tissue, we are met with the fact that the increased output of nitrogen during the water period accounts for less than one-fourteenth of the nitrogen which should have resulted from the disintegration of sufficient muscular tissue to have yielded the total creatine output. We arrive at this conclusion from the following calculation. The total excretion of creatine-nitrogen was 0.463 gram, which is equivalent to 1.45 grams of creatine. Supposing human muscle to contain 0.3 per cent. of creatine, we find that the disintegration of 483 grams of muscle would be necessary to liberate 1.45 gram of creatine. However, considering muscle to contain 20 per cent. of protein material we see that the disintegration of this 483 grams of muscle would

increase the output of total nitrogen 15.456 grams. Now our data from the water period show that this increase was only 1.079 gram. In other words, 14.377 grams or 93 per cent. of the 15.456 grams is unaccounted for. The explanation of this preponderance of creatine above the total nitrogen output may indicate that the creatine is held in such a combination within the muscle as to be released and excreted through the influence of factors which are not sufficiently active to cause complete muscular disintegration, as measured by total nitrogen output. We may logically infer, I take it, that three liters of water coursing through the body may well cause some such slight metabolic phenomenon as that necessary to account for the appearance of this creatine in the urine for a short period. That the usual metabolic rhythm is soon reëstablished, however, is indicated by the fact that creatine no longer appears in the urine as soon as the volume of urine excreted has been reduced to that customarily voided.

Dorner[16] has given us evidence that creatine may be released from a muscle in some such way as that suggested above. Whereas the normal content of creatine for rabbit muscle is from 0.428 per cent to 0.456 per cent., he found that sufficient creatine could be removed through starvation to reduce the creatine content to 0.356 per cent.

Mellanby[17] has also suggested a similar incomplete muscle breakdown followed by a nitrogen excretion which was inadequate to account for the complete disintegration of sufficient muscular tissue to have yielded the total creatine output. However, in the case of carcinoma cited by him, the conditions were not so striking as are those of our experiment, inasmuch as Mellanby could account for about one-half of the excreted nitrogen, whereas we can account for less than one-fourteenth on the basis of the creatine excretion.

From among the maze of hypotheses surrounding the metabolic relations of creatine and creatinine, we are unable to select one which seems more applicable in this connection than the one above mentioned. The fact that coincidently with the appearance of creatine in the urine there was noted a decrease in the output of

[16] Dorner, *Zeit. f. physiol. Chem.*, 1907, lii, 225.
[17] Mellanby, *Jour. of Physiol.*, 1908, xxxvi, 447.

creatinine is an observation which might perhaps be interpreted as indicating that these two substances have a common origin,[18] and that they represent two phases in the metabolism of a single substance.

From another standpoint we may consider that creatine represents a metabolic product which is formed in the liver[19] or other organs and under ordinary conditions is carried to the muscle and deposited. Creatinine then results from a part of the deposited creatine and is excreted. However, when the equilibrium of the metabolic cycle is disturbed, as it may very well be conceived to be when three liters of water above the volume usually ingested are introduced into the body during a period of twelve hours, the creatine is in part excreted without first passing to the muscle for deposit. At the same time, the creatinine output would decrease because insufficient creatine had been deposited in the muscle to furnish the requisite amount of creatinine to yield the normal output.

Again let us consider creatinine as the original substance formed in the liver.[20] Under ordinary conditions part of this creatinine is transformed into creatine and stored in the muscle. We can easily conceive in this connection that the flooding of the body with three liters of water may so stimulate the transformation of creatinine into creatine as to yield a lessened output of the former and an increased output of the latter, considering the mechanism of the muscle as inadequate to cope with the unexpected rush of creatine.

Doubtless some individuals will look upon the appearance of creatine in the urine under the influence of copious water drinking as indicating that the water has had a pernicious influence. However, we do not believe that our data will permit of any such interpretation. The careful and accurate investigation of the metabolic relations of creatine and creatinine is of comparatively recent birth. The mere fact that urinary creatine has always been a pathological token is no criterion that the normal human body may not be so

[18] Levene and Kristeller, *loc. cit.;* Lefmann, *loc. cit.*

[19] Mendel, *Science,* 1909, xxix, 584.

[20] Mellanby, *loc. cit.*

manipulated by normal means as to yield creatine in the urine. It would seem rather illogical to us to call a condition pathological when its only deviations from the so-called normal are (*a*) increased flow of digestive juices, (*b*) decreased output of feces, (*c*) decreased excretion of fecal nitrogen, both bacterial and non-bacterial, (*d*) better digestion of protein constituents of the diet, and (*e*) increased body weight.

INCOME AND OUTGO OF NITROGEN.

Data for the income and outgo of nitrogen are given in Table VI. Protein catabolism was so stimulated during the water period as to cause an increased output of urinary nitrogen amounting to an average of 0.215 gram per day. The feces of this period, however, contained but 0.973 gram of nitrogen per day as against 1.358 gram per day for the preliminary period. Thus it is probable that more of the nitrogen of the ingested food was utilized by the body during the water period (see discussion, p. 403). The nitrogen balance shows a gain of 1.656 gram of nitrogen during this period of five days, or a daily gain of 0.331 gram.

That the tendency for the body to store nitrogen was not limited merely to the time during which large amounts of water were being taken is shown by Table VI. During the final period of eight days, although the extra three liters of water which had been included in the daily menu of the water period, were eliminated, the tendency to store nitrogen continued. The data indicate that 9.246 grams of nitrogen were stored during an eight day interval, this being a daily gain of 1.156 gram. The tendency to gain nitrogen decreased as the period neared its end, however, and the final day showed a gain of but 0.451 gram, a value which is nevertheless about three times as great as the nitrogen balance of the last two days of the preliminary period. Evidently the ingestion of the large amount of water had so altered certain metabolic relations as to lead to increased nitrogen storing activity.

The point in connection with the income and outgo of nitrogen which is of the greatest practical interest is our observation of the pronounced decrease in fecal nitrogen during the period in which three liters of water were daily ingested with the meals. It seems

probable, therefore, that the protein constituents of the diet were more economically utilized during the period of extra water ingestion (see further discussion on p. 403). This finding is of particular interest when considered in connection with the recent investigation of Foster and Lambert.[21] These investigators worked with dogs provided with the Pawlow pouch and demonstrated conclusively that when water was added to the diet, there was a much more copious flow of gastric juice and that this gastric juice possessed a higher acidity than did the gastric juice which was poured forth upon a similar diet minus the water. In other words, water stimulated very markedly the secretion of gastric juice. Our results here reported finish the cycle and demonstrate the efficiency of this increased outpouring of highly acid juice. These two facts and the further observation that there was an increase in body weight and an improvement in the general mental and physical tone of the subject are sufficiently important observations to indicate the unquestionable benefits accompanying and following the drinking of large amounts of water with meals.

FECAL BACTERIA AND NITROGEN.

The data regarding these factors are shown in Table VIII. The method used for the quantitative determination of the fecal bacteria was a modification of Strasburger's method proposed by Dr. MacNeal of our university, as previously mentioned.

The conditions surrounding the experiment were such as to preclude the examination of the bacterial content of each individual stool. MacNeal's modification of Strasburger's method is very accurate but at the same time it is exceedingly time-consuming; therefore, with the large amount of chemical work necessary to be done in connection with this investigation and others running coincidently we were unable to make frequent bacterial examinations. The data, therefore, as given in Table VIII represent the results from three stools, one being examined during the preliminary period, one during the water period and one during the final period.[22] It is our purpose to study this feature farther in the

[21] Foster and Lambert, *loc. cit.*

[22] We are indebted to Mr. H. W. Hochmeister for assistance in making these determinations.

near future and to make an examination of each individual stool

Regarding the excretion of bacteria in the feces our data show that the daily output of dry bacteria was decreased under the influence of the copious water drinking. This fact is clearly shown by a daily excretion of 5.327 grams of dry bacteria during the preliminary period as compared with an excretion of 4.579 grams per day during the water period. This satisfactory change in the content of intestinal bacteria was further accentuated in the final period in which the bacteria value was reduced to an excretion of only 3.280 grams per day. The bacteria constituted 20.88 per cent. of the dry feces during the preliminary period, the much higher value of 24.37 per cent. during the water period, and they then assumed practically the original value 21.23 per cent. in the final part of the experiment. The bacteria as isolated yielded very similar nitrogen values.

Some interesting and very practical conclusions may be drawn from the data on fecal nitrogen. They indicate that the daily output of nitrogen by way of the feces was 1.358 gram during the preliminary period, and that under the influence of the daily ingestion of three liters of water this value was reduced to 0.973 gram. Apparently then the ingestion of this large amount of water caused a more economical utilization of the protein constituents of the diet. However when we consider our bacteria data in connection with our total nitrogen data we are much more impressed with the efficiency of the water diet. In the case of the preliminary period, for example, by subtracting the bacterial nitrogen from the total nitrogen, we note that 0.774 gram of nitrogen of non-bacterial origin was excreted daily. This value was reduced to 0.497 gram for the water period. There seems to us but one explanation for this condition of affairs. Evidently the water has brought about conditions which have caused a more rapid digestion and absorption of the protein constituents of the diet. This is borne out by the data showing a decrease from 0.774 to 0.494 under the influence of the water. There being less nitrogenous material present in the intestine during the water period, naturally the bacteria could not develop as satisfactorily as during the preliminary period. Therefore, we should expect to have less bacterial nitrogen

excreted daily, an expectation which we have fully verified in showing that 0.584 gram of this form of nitrogen was excreted daily during the preliminary period as against an excretion of 0.476 gram per day for the water period.

DIGESTION AND ABSORPTION OF PROTEIN.

During the preliminary period (Table V), the subject excreted 105 grams of fresh feces per day. Under the influence of the water ingestion the daily output was reduced to 74 grams, whereas the final period showed a somewhat higher fecal output than during the water period, but at the same time one which was somewhat lower than that in evidence during the preliminary period. This value was 98 grams per day. Calculated to a dry feces basis we find that 23 grams of dry feces were excreted per day during the preliminary period as against 17.5 grams for the water period and 20.4 grams for the final period. The dry matter in the feces was, therefore, decreased 24 per cent. through the copious water drinking.

Not only do we find less feces excreted during the water period, but we observe, as one would expect, a decreased nitrogen output also. From a daily excretion of nitrogen amounting to 1.358 gram during the preliminary period the daily output was decreased to 0.973 gram during the water period. In this connection it is interesting to note that, notwithstanding the fact that the dietary conditions were the same during the final period as those which were in force during the preliminary period, the daily output of nitrogen was only 1.043 gram, a value decidedly lower than that of the preliminary period and not far removed from that of the water period. This observation seems to indicate that the conditions brought about through the drinking of the large amount of water were not simply temporary, but that they were maintained with almost full force throughout the portion of the experiment which followed the drinking of the large amount of water. By calculation we find that the daily fecal nitrogen output was reduced 28.3 per cent. through the influence of the water ingestion, whereas the feces of the final period showed a nitrogen value 23.2 per cent. below that of the preliminary period.

A further fact of interest in this connection is that the feces

excreted during the water period contained less moisture than the fecal output of the preliminary period (Table V). The value is 78.1 per cent. for the preliminary period as against 76.3 per cent. during the time the large amount of water was ingested. In other words, the water content of the feces was inversely proportional to the amount of water ingested. If there was thus sufficient stimulation of the absorptive forces to leave less water unabsorbed from an ingestion of 3900 cubic centimeters than ordinarily occurred from an ingestion of only 900 cubic centimeters, it may well be that this new condition of affairs was also instrumental in bringing about a more rapid and complete absorption of the protein digestion products.

When we examine the data concerning fecal bacteria, we find an interesting situation. Table VIII shows that during the preliminary period there were 5.327 grams of dry bacteria excreted per day, whereas during the water period the daily output was only 4.579 grams. The water drinking had, therefore, apparently been instrumental in reducing the bacterial content of the feces 14 per cent. per day. It is also noteworthy that this reduction was not temporary, inasmuch as a still further reduction was obtained in the following period of the experiment. As was to be expected, the output of bacterial nitrogen followed quite closely the output of fecal bacteria, and registered a decrease of 18.5 per cent. in the water period below the preliminary period value.

In our opinion the data from the feces examinations are capable of a most far reaching and exceedingly important interpretation. Of course, we appreciate that we cannot safely make the nitrogen content of the feces a measure of food utilization. We are well aware that probably the larger portion of the non-bacterial nitrogen of feces is ordinarily made up of other material than food residues. We cannot, therefore, upon the basis of the total nitrogen data alone draw perfectly safe conclusions as to the economical utilization of the ingested protein.

Taking all factors into consideration, however, we believe that we are justified in drawing the conclusion that the protein constituents of the diet were more economically utilized during the period of

copious water ingestion. We base our conclusion upon four distinct observations as follows: (1) a decreased excretion of feces; (2) a lowered nitrogen output including both bacterial and non-bacterial nitrogen; (3) a lower moisture content in feces; (4) less bacteria in feces; (5) increased flow of digestive juices.

If we wish to apply a correcting factor to represent the fecal nitrogen due to metabolic products, we cannot do less than make the factor a constant, since a uniform diet was used. We cannot conceive of the metabolic products undergoing any pronounced variation under these conditions. The only time that the diet was at all modified was during the water period, at which time three liters of water were added to the daily diet. As to the influence of this unknown factor the experiments of Foster and Lambert previously mentioned have enlightened us. The water causes a pronounced stimulation of the gastric secretion resulting in the outpouring of an excess of gastric juice. This factor is directly responsible for an increased output of pancreatic juice, thus directly contributing to the feces an unusually high quota of metabolic products. Therefore, even if conditions surrounding the digestion and absorption of the ingested protein were identical from day to day throughout the experiment, with the exception of the water period, and if during this period a more copious supply of metabolic products were passed on to the feces we would expect the percentage of non-bacterial nitrogen to increase. When we examine the data, however, we note that during the preliminary period the feces contained 57.1 per cent. of non-bacterial nitrogen as against 50.9 per cent. of this form of nitrogen for the water period.

What apparently took place during the water period, as far as the digestive mechanism was concerned was that the entrance of the large amount of water into the stomach stimulated the flow of gastric juice thus facilitating the digestion of the protein constituents of the diet. The strongly acid chyme passing into the intestine caused a pronounced stimulation of the pancreatic secretion. The products of gastric digestion were consequently more rapidly and thoroughly digested. The coursing of the large volume of water

through the intestine walls so stimulated the absorption mechanism that the products of intestinal digestion were absorbed with increased rapidity and thoroughness. The intestinal bacteria, therefore, found less pabulum at hand, and in consequence these microorganisms were less well nourished than usual, a fact demonstrated by a decrease in the output of bacteria and of bacterial nitrogen. The effect of this stimulated digestion, absorption and assimilation, was noted at once in the increased body weight of the subject.

We realize that our experiment includes data collected from but a single individual and that the general application of the principle can only be shown by further tests upon other individuals. Other experiments are now under way along these lines. In the meantime, we shall consider the current statements regarding the exceedingly harmful influence of water drinking with meals as misleading.

CONCLUSIONS.

The daily drinking of three liters of water *with meals,* for a period of *five days,* by a man twenty-two years of age who was in a condition of nitrogen equilibrium through the ingestion of a uniform diet, was productive of the following findings:

1. An increase in body weight, aggregating two pounds in five days.

2. An increased excretion of urinary nitrogen, the excess nitrogen being mainly in the form of urea, ammonia, and creatine.

3. A decreased excretion of creatinine and the coincident appearance of creatine in the urine. The decreased creatinine output is believed to indicate that the copious water drinking has stimulated protein catabolism. The appearance of creatine is considered evidence that the water has caused a *partial* muscular disintegration resulting in the release of creatine, but not profound enough to yield the total nitrogen content of the muscle. The output of creatine is, therefore, out of all proportion to the increase in the excretion of total nitrogen.

4. An increased output of ammonia which is interpreted as indicating an increased output of gastric juice.

5. A decreased excretion of feces and of fecal nitrogen, the decrease in the excretion of fecal nitrogen being of sufficient magnitude to secure a lowered excretion of both the bacterial and the non-bacterial nitrogen.

6. A decrease in the quantity of bacteria excreted daily.

7. An increase in the percentage of total nitrogen appearing as bacterial nitrogen.

8. A lower creatinine coefficient.

9. A more economical utilization of the protein constituents of the diet.

10. The general conclusion to be reached as the result of this experiment is to the effect that the drinking of a large amount of water with meals was attended by many desirable and by no undesirable features.

TABLE I.

Urine Volume and Nitrogen Partition.

Date, 1909.	Day of experiment.	Urine volume.	Nitrogen.	Urea-nitrogen.	Ammonia-nitrogen.	Creatinine-nitrogen.	Creatine-nitrogen.
			Preliminary period.				
March.		c.c.	grams.	grams.	grams.	grams.	grams.
6	4	830	12.987	11.338	0.288	0.629	—
7	5	920	13.084	11.476	0.305	0.619	—
8	6	880	13.183	11.568	0.369	0.651	—
			Water period.				
9	7	3440	14.161	12.596	0.486	0.610	0.063
10	8	3840	13.491	11.583	0.499	0.616	0.024
11	9	3670	12.981	11.212	0.553	0.589	0.102
12	10	3610	12.976	11.455	0.485	0.608	0.055
13	11	4020	13.138	11.879	0.456	0.589	0.128
			Final period.				
14	12	1590	11.823	10.357	0.405	0.602	0.041
15	13	885	11.836	10.198	0.422	0.639	0.015
16	14	1025	12.925	11.169	0.369	0.586	0.035
17	15	890	11.946	10.299	0.374	0.642	—
18	16	830	12.017	10.374	0.333	0.629	—
19	17	870	13.030	10.835	0.344	0.659	—
20	18	930	12.905	11.293	0.325	0.627	—
21	19	1480	13.160	11.452	0.399	0.641	—

TABLE II.

Percentage of Total Nitrogen in the Form of Urea, Ammonia, Creatinine, and Creatine.

Date, 1909.	Day of experiment.	Total nitrogen.	Urea.	Ammonia.	Creatinine.	Creatine.
			Preliminary period.			
March 6	4	100	87.46	2.22	4.84	—
7	5	100	87.71	2.33	4.73	—
8	6	100	87.75	2.80	4.83	—
			Water period.			
9	7	100	88.95	3.43	4.31	0.44
10	8	100	85.86	3.70	4.57	0.18
11	9	100	86.37	4.26	4.54	0.79
12	10	100	88.49	3.74	4.69	0.42
13	11	100	90.44	3.47	4.48	0.97
			Final period.			
14	12	100	87.60	3.43	5.10	0.35
15	13	100	86.16	3.57	5.40	0.13
16	14	100	86.41	2.85	4.53	0.27
17	15	100	86.23	3.13	5.37	—
18	16	100	86.33	2.77	5.23	—
19	17	100	83.16	2.64	5.58	—
20	18	100	87.51	2.52	4.86	—
21	19	100	87.02	3.03	4.87	—

TABLE III.

Increased Nitrogen Excretion of the First Day of the Water Period as Compared with the Average Excretion of the Last Two Days of the Preliminary Period.

Determination.	Total Nitrogen.	Urea.	Ammonia.	Creatinine.	Creatine.
Increase in grams	1.027	1.074	0.149	—0.025	.063
Percentage increase	7.82	9.32	44.21	—3.94	—
Percentage increase as compared to the increase in total nitrogen	100.0	104.57	14.52	—2.43	6.13

TABLE IV.

Average Daily Increase in the Nitrogen Excretion of the Entire Water Period as Compared with the Last Two Days of the Preliminary Period.

Determination.	Total Nitrogen.	Urea.	Ammonia.	Creatinine.	Creatine.
Increase in grams	0.215	0.223	0.159	— 0.033	0.074
Percentage increase	1.64	1.94	47.18	— 5.20	—
Percentage increase as compared to the increase of total nitrogen	100.0	103.72	73.95	—15.35	34.42

TABLE V.

Weight and Nitrogen Content of Feces.

Period.	Length of Period.	Fresh feces.					Dry feces.			
		Total Weight.	Weight per day.	Moisture content.	Nitrogen.		Total weight.	Weight per day.	Nitrogen.	
					Total for period.	Weight per day.			Total for period.	Weight per day.
	days.	grams.	grams.	per cent.	grams.	grams.	grams.	grams.	grams.	grams.
Preliminary	6	630.1	105.0	78.1	8.147	1.358	138.0	23.0	8.147	1.358
Water	5	370.1	74.0	76.3	4.867	0.973	87.6	17.5	4.867	0.973
Final	8	783.9	98.0	79.2	8.344	1.043	163.1	20.4	8.344	1.043

TABLE VI.

Nitrogen Balance.

Period.	Length of period.	Nitrogen.				
		Income.	Outgo.		Gain or loss.	Average gain or loss per day.
		Food.	Urine.	Feces.		
	days.	grams.	grams.	grams.	grams.	grams.
Preliminary	2	29.308	26.267	2.716	+0.325	+0.163
Water	5	73.270	66.747	4.867	+1.656	+0.331
Final	8	117.232	99.642	8.344	+9.246	+1.156
Last day of final	1	14.654	13.160	1.043	+0.451	+0.451

TABLE VII.

Creatinine Coefficient.

Period.	Creatinine, milligrams.	Body-weight, kilos.	Creatinine coefficient.
Preliminary	631.0	70.96	8.89
Water	602.0	71.96	8.36
Final	620.0	72.17	8.70
Last day of final period	641.0	72.21	8.88

TABLE VIII.

Fecal Bacteria and Nitrogen.

Period.	Fecal bacteria.			Fecal nitrogen.				
	Weight.	Per cent. of dry feces.	Nitrogen.	Total weight.	Bacterial.		Non-bacterial.	
					Weight.	Per cent.	Weight.	Per cent.
	grams per day.		per cent.	grams per day.	grams.		grams.	
Preliminary	5.327	20.88	10.97	1.358	0.584	42.9	0.774	57.1
Water	4.579	24.37	10.40	0.973	0.476	49.1	0.497	50.9
Final	3.280	21.23	11.54	1.043	0.378	36.2	0.665	63.8

TABLE IX.

Body Weights, Volume and Specific Gravity of Urine.

Date, 1909.	Day of Experiment.	Body-weight.	Urine.	
			Volume.	Specific gravity.
		kilos		
PRELIMINARY PERIOD.				
March 3	1	71.69		1.024
" 4	2	70.82		1.030
" 5	3	70.23		1.030
" 6	4	70.63		1.030
" 7	5	71.09		1.0275
" 8	6	71.11		1.028
WATER PERIOD.				
March 9	7	71.42		1.008,
" 10	8	71.94		1.006
" 11	9	72.00		1.007
" 12	10	72.26		1.0075
" 13	11	72.18		1.007
FINAL PERIOD.				
March 14	12	72.17		1.016
" 15	13	72.06		1.025
" 16	14	72.21		1.025
" 17	15	72.24		1.026
" 18	16	72.15		1.027
" 19	17	72.11		1.027
" 20	18	72.29		1.0265
" 21	19	72.21		1.020

THE QUANTITATIVE CHANGES IN THE PROTEINS IN THE BLOOD PLASMA OF HORSES IN THE COURSE OF IMMUNIZATION.[1]

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When an animal is immunized against bacterial toxins (Seng,[2] Joachim,[3] Atkinson,[4] Ledingham[5]) and against foreign proteins (Moll[6]), there result certain characteristic quantitative changes in the proteins of the blood plasma. The content of serumglobulin is strikingly increased, even up to double the normal amount; at the same time, there is a diminution in the serumalbumin. Similar changes have been said to occur during starvation;[7] however, Moll has denied that the inanition is the cause of the globulin increase observed in precipitin sera.

Atkinson[8] showed that this serumglobulin increase is in some degree proportional to the antitoxic (diphtheria) potency of the serum. His analyses give figures for the protein precipitable on saturation with magnesium sulphate; this "globulin"[9] paralleled more or less the gross changes in the antitoxic potency in the serum for the one horse so examined. With his other observations on the increased "globulin" and the antitoxic content of antitoxic

[1] Received for publication January 18, 1910.

[2] Seng, *Zeit. f. Hyg.*, 1899, xxxi, 513.

[3] Joachim, *Arch. f. d. ges. Physiol.*, 1903, xciii, 558.

[4] Atkinson, *Jour. of Exper. Med.*, 1901, v, 67.

[5] Ledingham, *Jour. of Hyg.*, 1907, vii, 65.

[6] Moll, *Zeit. f. exper. Path. u. Therap.*, 1906, iii, 325.

[7] Glaessner, *Zeit. f. exper. Path. u. Therap.*, 1905, ii, 154.

[8] Atkinson, *Jour. of Exper. Med.*, 1901, v, 47.

[9] Under the classification of the proteins adopted by the American Physiological Society and the American Society of Biological Chemists, the greater part of the so-called serumglobulins are, at least for the horse, to be regarded as albumins.

serum, a more than incidental relationship between the serumglobulin change and the antitoxic content is suggested.

In a paper published while the present investigation was under way, Ledingham[10] gives results of the determination of the total protein, the serumglobulin and the serumalbumin in the course of immunization against diphtheria toxin. The serum was obtained at short intervals from two horses and a goat. With one horse, which failed to yield a high grade antitoxin, the serumglobulin content of the serum was essentially not increased over the normal. The other horse gave ultimately a 650 unit serum; the serumglobulin here had progressively increased along with the antitoxic potency. A goat serum which had reached 40 units showed a rise in both the serumalbumin and (to a much less degree) of the serumglobulin over the normal figures. From Ledingham's results on the two horses, a relation between the development of the antitoxic properties and the increase in the serumglobulin content is indicated.

The observations of Atkinson and of Ledingham, so far as we are aware, are the only determinations of the quantitative relation of the serumglobulin content and antitoxic potency throughout the course of immunization. The subject is of extreme importance because of the constant association of the anti-substance with the serumglobulin.[11] The serumglobulin increase in this protein during immunization may actually represent the accumulation of protective substances; at least if not identical, the antitoxin may be in some loose chemical combination with the protein. Mellanby[12] recently states, even, that the diphtheria antitoxin actually is a part of the serumglobulin (albumin α).

The poverty of the data as to the blood changes during immunization make additional and more conclusive experiments desirable when the significance of the problem as to the chemical nature of the protective substances is considered. In the present paper we present the results of a large number of analyses upon the protein changes in the plasma of eleven horses subjected to immunization.

[10] Ledingham, *loc. cit.*

[11] With the more soluble or "albumin" type of the serumglobulin.

[12] Mellanby, *Proc. of the Royal Soc.*, Series B, 1908, lxxx, 399.

We have followed quantitatively the protein changes in the sodium oxalate plasma in a series of eleven horses. At first, these were undergoing simultaneous immunization, under the direction of Dr. W. H. Park, to both tetanus and diphtheria toxins; the horses were subsequently kept on the toxin to which they responded best individually as regards antitoxin production.[13] The first few injections were made with toxin after the administration of antitoxin, as is the custom at the Research Laboratory. The immunization was forced, *i. e.*, the toxins were administered in relatively large doses, usually in weekly injections. The horses were bled for serum production at about 8, 10 or 14 day intervals, when a maximum antitoxic value was attained, as indicated by earlier test bleedings of about 500 cubic centimeters of blood. The regular bleedings for serum production (six to ten liters of blood) were instituted, accordingly, only *after* maximum antitoxic (and protein) changes had occurred, as shown in the tables. The variations during the period of increasing antitoxic potency, therefore, have not been influenced by severe hemorrhage.

Suitability of Individual Horses for Antitoxin Production.—As has long been known, certain young and healthy horses seem refractory for the production of diphtheria or tetanus antitoxins; again, certain horses will yield a much more potent antitoxin than others treated in an exactly identical manner. This idiosyncrasy is strikingly brought out in the behavior of the animals used in the present experiments. Thus six (319, 322, 323, 326, 327 and 328) of the eleven horses reacted to diphtheria but not to tetanus toxin; the plasma obtained from these six, at maximum potency, was 550 units per cubic centimeter or above, except in the case of 327 where the strength was 400 units. One horse (320) only gave a potent serum against the tetanus while simultaneously being refractory for the production of diphtheria antitoxin. Two horses (321 and 324) failed to react vigorously on immunization with either toxin; another (325) responded to some degree to both. Horse 318 was immunized to a high degree (600 units) to diphtheria toxin; transferred to tetanus, it yielded a very potent serum (300 units) and when returned to the diphtheria service almost the original potency was reëstablished. There is then an individual adaption on the part of the horse for the production of certain antitoxins; for other toxins the horse may be almost completely refractory.

[13] It was originally Dr. Park's intention to report this immunization experiment in detail. The present paper contains a description of the experiment and the results only in so far as is necessary to make our own report complete.

MAXIMUM ANTITOXIN POTENCIES IN UNITS.[14]

Horse.	Diphtheria.	Tetanus.	Horse.	Diphtheria.	Tetanus.
318	600	300	324	125	1
319	600	4	325	300	25
320	50	90	326	600	1
321	150	2	327	400	1
322	625	5	328	550	1
323	850	5			

Protein determinations were made in duplicate by heat coagulation and weighing on the oxalate plasma as follows:

1. The total protein.

2. The total protein less the fibrinogen.

The protein in an aliquot of the filtrate from the fibrinogen precipitated at 2.9[15] saturation ammonium sulphate and at an ultimate dilution (when precipitated) of tenfold the original volume of plasma taken for the fractioning.

3. The serum albumins.

The protein in aliquots of the filtrate at 5 (half saturation) ammonium sulphate at a dilution of the plasma, when precipitated, of tenfold the original volume of plasma taken.

4. (a) The serumglobulins soluble in saturated sodium chloride solution.

Separated by direct solution in saturated sodium chloride solution of the proteins precipitated at 5 or half saturation ammonium sulphate, at an ultimate plasma dilution of 1 : 10.

(b) Or the protein in solution on saturating the plasma with sodium chloride.

The several times diluted plasma was saturated with the dry salt and made up to ten times the volume of plasma taken with saturated sodium chloride solution. Determinations were made on aliquots of the filtrates. This separation was more commonly used than the above.

5. The "pseudoglobulin" plus the serum albumin.

[14] Maximum potencies for diphtheria and tetanus antitoxins, of course, are not always here coincident.

[15] The degree of saturation, as here expressed, indicates a concentration in 10 cubic centimeters of the precipitated mixture of 2.9 cubic centimeters of saturated ammonium sulphate solution. The misconceptions that arise from the use of "per cent. saturation" and even from "third saturation" and "half saturation" have already been discussed in a previous paper. Banzhaf and Gibson, *Jour. of Biol. Chem.*, 1907, iii, 254.

The protein of aliquots of the filtrate from the plasma precipitated at third (3.3) saturation ammonium sulphate and at ultimate dilutions of 1.5, 5 and 10 times the volume of the plasma used.

This volume of the precipitates obtained in the above determinations was so small that but little error should be obtained in employing aliquots of the filtrates as above for the analyses. In the precipitation of the "euglogulin" at 1:1.5 dilution, however, such error must influence the results somewhat; even, here, we were never dealing with a precipitate from 10 cubic centimeters of oxalate plasma of over 0.5 gram in a volume, when precipitated, of 15 cubic centimeters. Inasmuch as the density of proteins is greater than water, and the volume of solvent is increased somewhat by their solution, the error in the analytical results for the "euglobulin" at the dilution of the plasma of 1:1.5 must certainly be less than 3 per cent. This error is perfectly negligible in the interpretation of the figures for the "euglobulin."

From the analytical data so obtained, the content of the plasma in the following protein constituents is available.

The total protein.
Fibrinogen.
Serumalbumins[16]
Serumglobulins[16]
Serumglobulin insoluble in saturated sodium chloride solution.
Serumglobulin soluble in saturated sodium chloride solution.
"Euglobulin" precipitated at ultimate dilutions of the plasma of 1:15, 1:5 and 1:10.
"Pseudoglobulin," similarly precipitated.

Because of the large amount of analytical data obtained, it does not seem advisable to go into details of the variations in each of the eleven horses examined. Certain interesting and important *general* features of the results will be discussed in full and examples given from the material at hand. The conduct of the experiment and the results of the analyses are more fully given in the tables at the end of this article. Some graphic representations of the protein and antitoxic changes are also reproduced.

The characteristic serumglobulin increase in the plasma during immunization was observed *in all the eleven horses.* Certain char-

[16] By "serumalbumins" is meant the protein material commonly known as such and consisting probably of more than a single individual substance. Similarly, the "serumglobulins" refer to the remaining proteins of the plasma, excepting the fibrinogen.

acters of this increase may be discussed. The maximum increase in the serumglobulin was usually coincident with the maximum antitoxic potency of the plasma. Two exceptions were noted (Horses 320 and 324). The maxima were attained in the seventh to the ninth week of immunization, but both antitoxin and serum globulin may be diminished even at the time of the first antitoxin bleeding[17] (319, 322, 323, 325).

The curves of the serumglobulin increase and the development of the antitoxic potency were not otherwise parallel. The maximum serumglobulin content in two horses (320 and 324) was actually reached previous to the highest antitoxic potency.

After immunization for a period of 39 days, Horse 320 gave a plasma of 20 antidiphtheria units per cubic centimeter, the serumglobulin content of which was increased 42.9 per cent. over the normal. The plasma obtained on the fifty-ninth day tested up 35 antidiphtheria and 2 antitetanus units; the serumglobulin had increased to its maximum of 87.6 per cent. over the normal. Twenty-eight days later the potency had risen to 50 units for each antitoxin, while the serumglobulin increase had fallen to 69.7 per cent. Subsequently, with a further rise in the tetanus antitoxin to 90 units, the serumglobulin had diminished to 48.4 per cent. over the normal. For the second horse, 324, antitoxic potencies of 25, 125 and 126 units were accompanied by a serumglobulin content above the normal of 59.3 per cent., 48.5 per cent. and 38.9 per cent., respectively.

With the exception of Horses 318 and 319, on which we have no "intermediate" analyses, the other animals all showed a marked increase in the serumglobulin content, *preceding* the development of the antitoxic properties.

For example, 32 days after the combined immunization was started, Horse 322 yielded a plasma which tested 50 units of diphtheria antitoxin per cubic centimeter and in which the serumglobulin had been increased 44.8 per cent.; 35 days later, the plasma tested 625 units, and the serumglobulin increase was 90.6 per cent. The antitoxic potency, therefore, had been brought up over 12 times while the serumglobulin increase had been only double during the same period. Similarly, the serumglobulin in the plasma of Horse 325 had increased 40.7 per cent. after 27 days' immunization; the potency being just 5 units; on the fifty-fourth day, the serumglobulin increase was 90.2 per cent., while the antitoxic potency had risen to 600 units of diphtheria and 1 unit of tetanus antitoxin.

[17] The analyses for the plasma obtained at the first "full" bleeding are discussed with the earlier or "test" bleedings inasmuch as the horses had not previously been subjected to the severer hemorrhage.

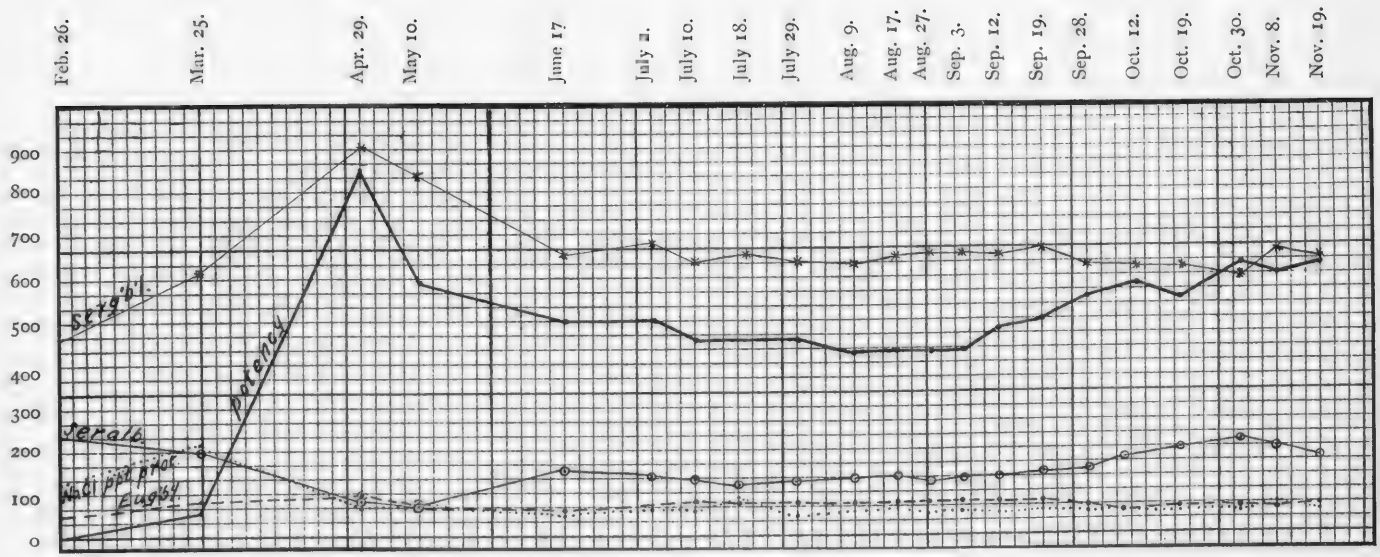

FIG. 1. Antitoxic and protein variations in the blood plasma of horse 323. The numerals to the left represent percentages of protein and the antitoxic potency in units. The dates of the bleedings are given above the tracing. A star (*) represents the first regular antitoxic or "full" bleeding.

It should be remarked that maximum antitoxic potency does not necessarily mean that the other immune substances are present in greatest amounts or that these are developed in corresponding degree with the antitoxin. Immunization aimed to be of specific character may lead to the production of other antibodies. Dr. K. R. Collins[18] has shown that group and specific bacterial agglutinins

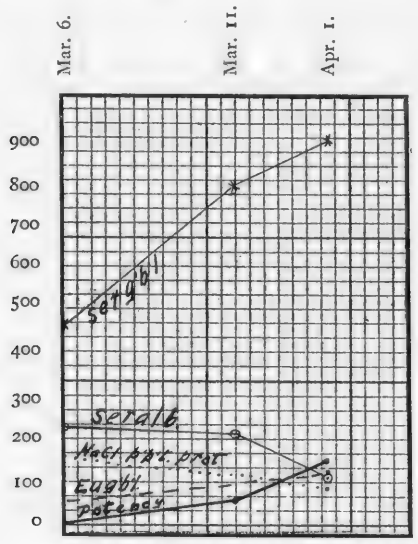

FIG. 2. Horse 321.

can be developed by immunization against yeasts, enzyme preparations and nucleins; these experiments afford actual proof of the development of many widely different anti-substances during immunization. A similar stimulation leading to the formation of numerous antibodies, besides the precipitins and specific antitoxins, probably occurs on immunization with bacterial toxins. Such substances may represent the increase in the more soluble serum globulin, with which fraction they are associated.

While the greatest rise in the serumglobulin was usually coincident with maximum antitoxic potency, as already pointed out, the

[18] Collins, *Jour. of Exper. Med.*, 1908, x, 529.

extent of this increase in the serumglobulin was practically independent of the antitoxic potency when the results on more than one horse were contrasted. There may be, then, no relation between

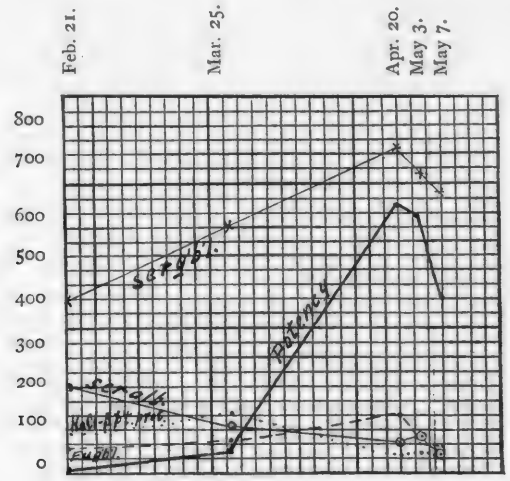

FIG. 3. Horse 322.

the absolute or percentage increase of the serumglobulin and the antitoxic potency in the plasma of different horses. The increase in the serumglobulin of refractory horses may surpass that in the plasma of some of those yielding a high antitoxin. The greatest absolute increase in serumglobluin (4.40 grams), however, was observed in the plasma of the horse (323) which supplied the strongest antitoxic serum of the series. In contrast, the refractory horse (321) showed an absolute increase in the serumglobulin of 4.07 grams per 100 cubic centimeters of plasma.

For Horse 318, this increase was 41.1 per cent. (2.01 grams per 100 cubic centimeters of plasma). For Horse 319, treated in exactly the same manner during immunization and yielding a plasma of the same potency (600 units diphtheria), the increase was 86.3 per cent. (3.66 grams). Horse 321 showed an increase of 87.6 per cent. (4.07 grams) in the serumglobulin when supplying a plasma of maximum potency of only 150 units per cubic centimeter; this horse failed to react well to the toxin not only as regards antitoxin production, but died subsequently of toxemia when the immunization was continued. Horse 323 gave a maximum of 850 units (diphtheria) and the serumglobulin had been

increased 95.6 per cent. with the greatest absolute increase (4.40 grams) we have observed. Horse 328 yielded a 550 unit plasma, the serumglobulin increase being 113.9 per cent. (4 grams). The refractory horse (324) is especially interesting. At the third bleeding (May 31, '07) a potency of 125 units (diphtheria) and a serumglobulin increase of 48.5 per cent. (2.69 grams) were found; 19 days later (June 19, '07) the antitoxic strength was unchanged, but the serumglobulin content had fallen until the increase was only 38.9 per cent. above the normal. A similar change was observed for Horse 326, where the serumglobulin maximum fell from 7.61 grams (March 30, '07) to 5.92 grams

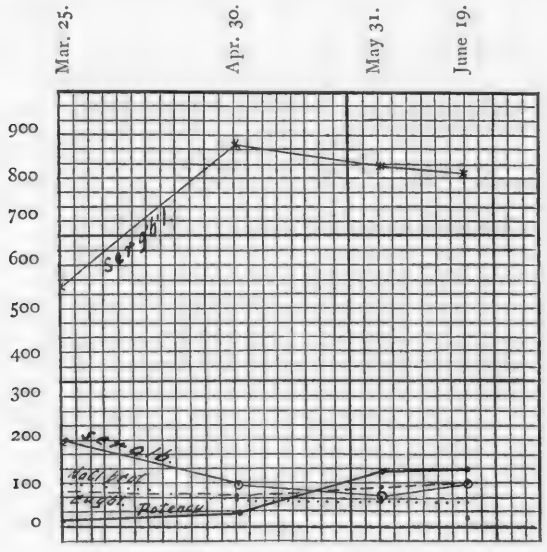

FIG. 4. Horse 324.

without change of antitoxic strength; the results here, however, are complicated by the "full" bleeding.

When the serumglobulin had reached a maximum with maximum antitoxic strength, the protein diminished with the decrease in potency even before regular bleedings were established. It was the practice in the Research Laboratory to bleed the horses for antitoxin production when test bleedings indicated a high or maximum potency. In some instances, the horses reached a maximum and the antitoxic potency had fallen somewhat by the time the first regular "full" bleeding was instituted. This diminution in potency

SERUMGLOBULIN INCREASE AT MAXIMUM ANTITOXIC POTENCY.

Horse.	Potency.		Serumglobulin increase.	
	Diphtheria.	Tetanus.	In gms. per 100 c.c. of plasma.	Per cent.
318	600	1	2.01	41.1
319	600	1	3.66	86.3
320	50	90	2.19	46.2[20]
321	150[19]	2	4.07	87.6
322	625	5	3.56	90.6
323	850	5	4.40	95.6
324	125[19]	1?	2.69	48.5[20]
325	300	4	3.22	89.1
326	600	1	3.61	90.2
327	400	1	2.87	102.1
328	550	1	4.00	113.9

was accompanied by a lowering of the serumglobulin from the maximum content. This fall, however, seems to have had no quantitative relation to the degree of the potency changes which in some instances were very slight. Accordingly, the lowering of the serumglobulin is probably identical with the submaximal fall in serumglobulin coincident with the highest potency in the plasma of Horses 320 and 324, and is characteristic in itself and more or less independent of the antitoxin variations.

For instance, Horse 319 gave a maximum of 600 units of diphtheria antitoxin and 1 unit of tetanus. The serumglobulin increase was then 3.66 grams per 100 cubic centimeters of plasma. With a change in antitoxic potency to 550 units of diphtheria and 2 of tetanus antitoxin (bleeding April 2, '07), the serumglobulin increase had fallen to 2.81 grams. Thus for a decrease in the antitoxin properties of about one-twelfth, the serumglobulin increase had been reduced one-fourth. A fall in the potency of the plasma of Horse 322 from 625 units of diphtheria antitoxin and 5 of tetanus to 600 units of diphtheria (May 3, '07) cut down the increase in the serumglobulin from 3.56 to 3.01 grams. Here, then, a fall of one-twenty-fourth in potency caused a drop in the increase of serumglobulin of a trifle less than one-sixth. The plasma of Horse 323 fell from a maximum of 850 units of diphtheria and 5 of tetanus antitoxin (April 29, '07) to 550 units of diphtheria (May 10, '07); the drop of over 35 per cent. in the potency was accompanied by a curtailment of 22.5 per cent. in the increase of the serumglobulin over the normal.

We have already shown that during immunization the serumglobulin increase tends to precede the development of the antitoxic potency; that the two reach a maximum, usually coincident, and

[19] Refractory horse.

[20] Maximum serumglobulin increase preceded maximum antitoxic potency.

that with the fall in potency, or even before, the increase in the serumglobulin is diminished to greater degree than the potency change. This last decline continued for one to three "full" bleedings, apparently irrespective as to whether the antitoxic properties were maintained or not. Subsequently there was a slight increase in the serumglobulin.

For example, at the first "full" bleeding (April 30, '07) of Horse 326, the 8 liters of plasma obtained tested 600 units per cubic centimeter, and the content in serumglobulin, 7.61 grams per 100 cubic centimeters. On the next "full" bleeding (May 10, '07), the serumglobulin content of the plasma, of the same potency, had fallen to 5.92 grams. We are not dealing here with the effects of dilution, through bleeding, because the serumalbumin has been increased from 1.16 to 1.44 grams per 100 cubic centimeters of plasma. Eight days later (May 18, '07) the potency was still 600 units; the serumglobulin content had increased from 5.92 grams to 6.96 grams per 100 cubic centimeters of plasma, while the serumalbumin had fallen from 1.44 grams to 0.98 gram. Practically the same fall is shown by the analyses in the plasma of Horses 319, 320, 323 and 325. For Horse 318, similar variations occur, but because of the double immunity here it is impossible to give a comparative value to the potency variations. The later rise is well brought out in the figures for Horses 318, 319, 323 and 325.

The serumglobulin content was maintained high and fairly constant during active antitoxin production. At times there was a tendency for the serumglobulin to follow quantitatively the potency variations; at other times, the increase was independent of the antitoxin changes (Horse 326, May 19, '07 and June 13, '07). With Horse 325, for instance, the serumglobulin did not increase for a rise in potency from 150 to 250 units (July 2, '07 to Sept. 17, '07) in the course of the regular bleedings, but was diminished from 6.09 to 5.39 grams. per 100 cubic centimeters of plasma. Subsequently when the lowering of the serumglobulin content was accompanied by a corresponding drop in the antitoxic potency, interruption of the regular "full" bleedings failed to check the diminution of either potency or serumglobulin.

In the course of immunization, then, there is a marked increase (even over 100 per cent.) in the serumglobulin content of the blood plasma. This increase tends to precede the development of maximum antitoxic potency and is quantitatively independent of the latter. With a diminution of the potency, the serumglobulin increase is reduced, with a tendency for this change to precede the

antitoxic variation; then after a slight rise, the content of serumglobulin is maintained at a fairly constant level in spite of repeated "full" bleedings at frequent intervals. The serum globulin now may or may not be independent of incidental variations in the antitoxic potency. As a rule, it does parallel to some extent the pronounced changes in the antitoxic properties of the plasma.

Changes in the "euglobulin" must be considered because the increase during immunization of the serumglobulin has been stated by Joachim[21] to take place in this fraction. Some discussion of the chemistry of the serumglobulin fractions is desirable.

The serumglobulin is commonly stated to consist of at least two different proteins, termed the "euglobulin" or true globulin and a more soluble protein, the "pseudoglobulin." The "euglobulin" is supposed to be insoluble in pure water, and accordingly precipitable on dilution or dialysis; it is thrown down also by slight acidification. The "pseudoglobulin belongs by definition to the group of true albumins, *i. e.,* it is soluble in pure water. It has received the title "pseudoglobulin" probably because the earlier differentiation was made on the basis of precipitation on saturation of its solution with magnesium sulphate. The separation of the two proteins then should be relatively easy by the dialysis method. It had always been our experience, however, that the ordinary globulin precipitation methods, with the exception of the salting out reactions, are practically without effect on horse serum. On dilution, dialyzation,[22] or slight acidification of the natural or previously neutralized (phenolphthalin) horse serum, only a very small amount of precipitate is obtained. In practice, investigators have commonly used the fractional precipitation by salting out with ammonium sulphate. A half volume of the saturated ammonium sulphate solution is added to the serum; the precipitate is the "euglobulin." The separation is not sharp at all. The filtrate contains the serumalbumin and "pseudoglobulin"; the latter can be thrown down by the further addition of saturated ammonium sulphate solution to "half" saturation, *i. e.,* until the mixture con-

[21] Joachim, *loc. cit.*

[22] Long continued dialysis may lead to denaturalization and precipitation of otherwise soluble proteins; more than forty-eight hours is hardly necessary for the separation of typical albumins and globulins.

tains equal volumes of serum and the salt solution. The "euglobulin" may be further purified by redissolving the precipitate in a measured quantity of water and again adding a half volume of the saturated ammonium sulphate solution. In current writings the identity of this "fractioned" euglobulin has been confused with the slight precipitate obtained on dialysis, dilution or slight acidification, in spite of the early work of Freund and Joachim.[23]

The differentiation of the serumglobulin into "eu" and "pseudo" fractions by the ammonium sulphate salting out was further established by the results of E. P. Pick.[24] This investigator fractioned various antitoxic, agglutinative and other sera and differentiated the antibodies chemically according to the fraction with which these were precipitated. Others[25] have also similarly fractioned individual antisubstances of various kinds and reported with which fraction their own particular body was associated. One of us (with Dr. K. R. Collins)[26] has already shown that no such separation of the agglutinins in polyagglutinative sera can be made, and has failed to verify Pick's results on antitoxic sera.[27] We[28] have, however, found that the more soluble of several fractions of the "pseudoglobulin" are richest in antitoxin per gram of protein. By employing the ammonium sulphate fractioning, Ledingham,[29] like Joachim, found that the increase in serumglobulin affected the "euglobulin" more than the "pseudoglobulin" fraction, *i. e.*, the great increase in the serumglobulin took place in the non-antitoxic portion. Ledingham's interpretation of his results, as regards this point, seems inconclusive, for he does not grasp the significance and understand the limitations in the salt fractioning of proteins.

[23] Freund and Joachim, *Zeit. f. physiol. Chem.*, 1902, xxxvi, 407.

[24] Pick, *Beit. z. chem. Physiol. u. Path.*, 1901, i, 351.

[25] Fuld and Spiro, *Zeit. f. physiol. Chem.*, 1900, xxxi, 133; Landsteiner, *Cent. f. Bakt., Orig.*, Abt. I, 1900, xxvii, 357; Jacobi, *Beit. z. chem. Physiol. u. Path.*, 1901, i, 51; Cathcart, *Jour. of Physiol.*, 1904, xxxi, 497; Glaessner, *Beit. z. chem. Physiol. u. Path.*, 1904, iv, 79; Simon, Lamar and Bispham, *Jour. of Exper. Med.*, 1906, viii, 651; Opie and Barker, *ibid.*, 1907, ix, 207.

[26] Gibson and Collins, *Jour. of Biol. Chem.*, 1907, iii, 233.

[27] Ledingham (*loc. cit.*) also states that the diphtheria antitoxin in goat serum is not invariably linked to the euglobulin fraction as maintained by Pick.

[28] Banzhaf and Gibson, *loc cit.*

[29] Ledingham, *loc. cit.*

We have already stated in an earlier paper that precipitation limits in the salting out of proteins are characteristic only in so far as they should be interpreted as well from the standpoint of the *solubility* of the protein in certain amounts of the salt solution of a definite concentration. Precipitation occurs because the solvent is saturated with the protein, *i. e.,* it can hold no more in solution; with a greater volume for the solvent more precipitated protein will go into solution. Moreover, other kinds of proteins remaining in solution probably affect the precipitation of the less soluble forms. Haslam's[30] criterion of purification calls for repeated precipitation at the same volume until the organic nitrogen in the filtrate is constant.

The observations associating the increase in the serumglobulin with the questionable "euglobulin" fraction have been made by direct precipitation of the serum with half its volume of saturated ammonium sulphate. Where reprecipitated for purification, the protein has usually been redissolved to the original volume and the same amount of ammonium sulphate solution added as before. Inasmuch as the serumglobulin content may have been doubled as the result of immunization, would not a great part of this increase be found naturally in the "euglobulin" fraction since solubility of the protein must be considered? The precipitate obtained at "one-third" saturation is not free from antitoxin.[31] It may contain the greater part of it when the undiluted serum is precipitated by the addition of a half volume of saturated ammonium sulphate solution.

Ten cubic centimeters of the normal plasma were precipitated at one-third saturation (3.3) by the direct addition of 5 cubic centimeters of the saturated ammonium sulphate solution; again 10 cubic centimeters of the plasma were diluted to 33⅓ cubic centimeters and precipitated by the addition of 16⅔ cubic centimeters of the saturated ammonium sulphate solution, and finally, a third 10 cubic centimeters of the plasma were diluted to 66⅔ cubic centimeters and precipitated with 33⅓ cubic centimeters of the saturated salt solution. We had then ultimate dilutions to 15, 50

[30] Haslam, *Jour. of Physiol.,* 1905, xxxii, 267.

[31] Brieger, *Festschrift für R. Koch,* Jena, 1903, p. 445.

and 100 cubic centimeters. The precipitates were allowed to stand for some hours, with occasional shaking, to permit mechanically precipitated material to return into solution. The "euglobulin" at the three dilutions was determined as outlined earlier in the paper.

In such an experiment with Horse 319, the normal total serumglobulin (4.24 grams per 100 cubic centimeters of plasma) was found to include 2.43, 0.79 and 0.48 grams of "euglobulin," according as the dilution of the precipitated plasma was 1 : 1.5, 1 : 5 or 1 : 10. When similarly fractioned at the height of immunization the three "euglobulins" were 5.39, 1.78 and 0.98 grams at the three respective dilutions. Of the increase of 3.66 grams in the total serumglobulin, about four-sevenths (2.96 grams) would be contained in the "eu" fraction at the 1 : 1.5 dilution; 0.99 grams, or about two-sevenths, would be accounted for at the 1 : 5 dilution, and but one-seventh at the 1 : 10 dilution. The precipitations were probably influenced by the lowered serumalbumin content of the antitoxic plasma. It will be noted that the "euglobulins" obtained at the three ultimate dilutions have about the ratio 5 : 2 : 1 for almost any of the plasma samples analyzed.

A similar behavior on the part of the "euglobulin" at the several dilutions was observed in the analyses for each horse. Most of the "euglobulin" determinations during the routine "full" bleedings were made at the 1 : 10 dilution only, and these figures probably most fairly represent the content of this protein, if such actually exists. Throughout the whole series of experiments, the percentage of increase in the "euglobulin" tended to parallel that of the total serumglobulin.

Because of the unsatisfactory characterization of the "eu" and "pseudo" globulin by the ammonium sulphate fractioning, it seemed of interest to differentiate in some modified way the relative serumglobulin quantitative changes. We have accordingly determined that portion of the serumglobulin precipitated on saturation with sodium chloride. Freund and Joachim state that saturation with sodium chloride precipitates all the "euglobulin," but that this includes only a part of the product obtained at 3.3 saturation ammonium sulphate. We may consider that we have as sharp a differentiation as possible between the two serumglobulins; of course, the

individuality of each cannot yet be asserted. The sodium chloride separation probably more truly represents the common conception of the euglobulin" and "pseudoglobulin," though the ammonium sulphate fractioning heretofore has usually been employed.

A glance at the table of analyses for any bleeding will show that the serumglobulin precipitated on saturation with sodium chloride and the ammonium sulphate "euglobulin" are quantitatively different. Accepting for the "euglobulin" the figures obtained with the ammonium sulphate at 1:10 dilution, we found that the sodium chloride gave a precipitate of serumglobulin in the normal plasma that was from two to three times greater than the corresponding fractioned protein.

The changes during the immunization in the saturated sodium chloride precipitated serumglobulin are significant. With the rapid rise in the serumglobulin content after immunization has been started, there was observed a very considerable increase in the portion of the serumglobulin precipitated on saturation with sodium chloride. This rise is shown in the record for Horses 322, 323, 325 and 327, but was not observed with the others.[32] Subsequently there was a sharp fall in the sodium chloride precipitable serumglobulin. This decrease was maintained far below the absolute and per cent. normal, and tended to vary inversely with the total serumglobulin content; the behavior in this respect is exactly the opposite of the "euglobulin" fractioned with ammonium sulphate, and parallels closely the changes in the serumalbumin.

For example, the serumglobulin precipitated on saturation with sodium chloride, in the normal plasma of Horse 325 was 0.91 gram per 100 cubic centimeters of plasma, or 24.7 per cent. of the total serumglobulin (3.68 grams). With the preliminary rise of 36.7 per cent. in the total serumglobulin, the sodium chloride "euglobulin" had increased over 60 per cent. above the normal (1.54 grams per 100 cubic centimeters of plasma) and to 30.6 per cent. of the total serumglobulin. The serumglobulin precipitable on saturation with sodium chloride then sharply fell; with the coincident maxima of antitoxic potency and total serumglobulin content, this sodium chloride "euglobulin" was only 0.49 gram (per 100 cubic centimeters of plasma) or 7.1 per cent. of the total globulin. At the same time, the ammonium sulphate "euglobulin" had increased from a normal of 0.42 gram to 1.00 gram per 100 cubic centimeters of plasma.

[32] On Horses 318 and 319 there were no intermediate test bleedings.

The variations in the content of serumalbumin during immunization have already been described in detail by Atkinson and by Ledingham. We have also found the curious and characteristic diminution in the content of the plasma in this protein. The minimum content is reached, as a rule, somewhat subsequent to the time of the serumglobulin and antitoxic maxima; it occurred usually during the period of the routine bleedings. While the greatest increase observed in the total serumglobulin was about 114 per cent., the serumalbumin may be reduced to less than 20 per cent. of the normal —a diminution from 2.80 grams to 0.52 gram being observed in the case of Horse 319. The course of the plotted curve of the serumalbumin then was roughly the reverse of that of the total serumglobulin; the serumalbumin, however, tended to return to the higher level when the horse had been in use for several months as an antitoxin producer (319, 323, 325).

The behavior of the sodium chloride "euglobulin" and the serumalbumin suggests that these proteins are sacrificed in order to compensate for the great absolute increase in the more soluble serumglobulin with which the anti-properties are associated. Possibly because of the increasing viscosity, there must be a physiological limit to the amount of protein which can be carried in the blood plasma. Some eighty odd determinations have indicated that this limit is attained when the protein content is between ten and eleven grams per hundred cubic centimeters of plasma.

Moll[33] has suggested that this serumalbumin is chemically transferred over into the serumglobulin during immunization. This statement is based on his heat and alkali transformation of albumin into globulin (?). Are we to accept, however, that the sodium chloride "euglobulin" might similarly be changed over into the more soluble serumglobulin?

The changes in the fibrinogen were often sudden and there was no apparent association with antitoxic potency, serumglobulin content or with bleeding. Analyses gave figures for fibrinogen of from 0.21 gram to 1.0 gram per 100 cubic centimeters of plasma, with variations in the individual horse of about 0.5 gram.

SUMMARY.

Gravimetric determinations were recorded for the total and several individual proteins (in the sodium oxalate plasma) fractioned

[33] Moll, *Beit. z. chem. Physiol. u. Path.*, 1904, iv, 563.

with ammonium sulphate and sodium chloride. At precipitation, the plasma salt mixture had been diluted to a final volume of ten times the amount of plasma employed. Coagulations were on aliquot portions of filtrates, and the individual protein constituents (except serumalbumin) were calculated by difference.

The eleven horses had been subjected to simultaneous immunization against diphtheria and tetanus toxins, each horse being subsequently continued on the toxin to which it responded best. Test bleedings of about 500 cubic centimeters only were made until maximum antitoxic potency (with almost coincident greatest variation

PROTEIN VARIATION DURING IMMUNIZATION.[34]

Horse 318.

Date of bleeding, 1907.	Last amount of diphtheria toxin injected about seven days before date of bleeding.	Last amount tetanus toxin injected about seven days before date of bleeding.	Diphtheria units per cubic centimeter.	Tetanus units per cubic centimeter.	Amount plasma recovered from bleeding.	At dilution 1:10; total protein.	At dilution 1:10; fibrinogen.	At dilution 1:10; serum albumin.	At dilution 1:10; serum globulin.	At dilution 1:10; sodium chloride insoluble globulin.	At dilution 1:1.5; euglobulin.	At dilution 1:5; euglobulin.	At dilution 1:10; euglobulin.
Jan. 24	Normal				400			2.83	4.89	1.68	3.26	1.18	0.65
March 11	375	160	600	1	400	9.35	0.48	1.97	6.90	1.44	4.68	1.78	0.98
April 2	475	250	550	10	7300	8.97	0.32	1.95	6.70	0.90	4.84	1.80	0.96
" 10	600	375	500	70	7700	8.32	0.42	1.84	6.06	0.45			0.90
" 24	Diphtheria-toxin discontinued	900	400	110	7850	8.04	0.56	1.47	6.01	0.84	4.31	1.55	0.79
May 14		1200	200	125	7060	8.89	0.68	0.97	7.24	0.55	4.89	1.80	0.96
" 20			125	160	6070	8.15	0.53	0.89	6.73	0.69			0.99
June 8		1000	50	300	5000	8.18	0.31	1.08	6.79	0.73			0.84
July 3		1200	10	250	6400	7.90	0.27	1.27	6.36	0.70	4.82	1.79	0.96
" 10			4	200	6300	7.54	0.34	1.25	5.95	0.57			
Sept. 6	400[36]	Tetanus toxin discontinued	200		400	8.11	0.40	1.30	6.41	0.75			0.99
" 25	560		400		5900	8.20	0.35	1.34	6.51	0.60	4.77	1.75	0.97
Oct. 9	600		450		6000	8.19	0.27	1.50	6.42	0.58			1.00
" 19	650		500		5900	8.30	0.30	1.66	6.34	0.62			0.96
" 30	700		550		6100	8.60	0.21	1.86	6.53	0.59			0.93
Nov. 8	750		550		6300	8.77	0.24	1.88	6.65	0.75			1.00
" 19[35]	750		575		6000	8.72	0.30	1.82	6.60	0.73	4.56	1.68	0.87

[34] Analyses are given as grams of coagulable protein per 100 cubic centimeters of plasma.

[35] Subsequent bleedings were as follows: Nov. 27, '07, 450 units, 6,100 cubic centimeters; Dec. 6, '07, 450 units, 5,850 cubic centimeters; Dec. 16, '07, 450 units, 5,800 cubic centimeters; Dec. 27, '07, 400 units, 6,200 cubic centimeters; Jan. 6, '08, 350 units, 6,200 cubic centimeters, and Jan. 7, '08, 200 units, 8,000 cubic centimeters. The horse died while bleeding on Jan. 7, '08.

[36] Diphtheria toxin injections resumed Aug. 14, '07.

PROTEIN VARIATION DURING IMMUNIZATION.[37]

Horse 319.

Date of bleeding, 1907.	Last amount of diphtheria toxin injected about seven days before date of bleeding.	Last amount of tetanus toxin injected about seven days before date of bleeding.	Diphtheria units per cubic centimeter.	Tetanus units per cubic centimeter.	Amount of plasma recovered from bleeding.	At dilution 1:10; total protein.	At dilution 1:10; fibrinogen.	At dilution 1:10; serum albumin.	At dilution 1:10; serum globulin.	At dilution 1:10; sodium chloride insoluble globulin.	At dilution 1:1.5; euglobulin.	At dilution 1:5; euglobulin.	At dilution 1:10; euglobulin.
Jan. 24	Normal				400			2.80	4.24	1.29	2.43	0.79	0.48
March 11	375	160	600	1	400	9.32	0.43	0.99	7.90	1.10	5.39	1.78	0.98
April 2	475	250	550	2	7100	8.55	0.33	1.17	7.05	0.82			1.00
" 10	600	375	525	4	8000	8.51	0.43	1.25	6.83	0.50	5.15	1.89	1.00
" 18	650		550		7850	7.85	0.52	0.87	6.46	0.69			0.88
" 27	700		575		5600	8.66	0.71	0.89	7.06	0.69	5.75	2.00	1.10
May 7	700		550		8000	8.02	0.61	0.81	6.60	0.49			0.90
" 14	750		550		8700	8.20	0.82	0.52	6.86	0.57			0.98
" 23	750		550		7900	8.66	0.74	0.55	7.37	0.67			0.99
June 1	850		550		6400	8.86	0.76	0.75	7.35	0.56			1.02
" 10	850		550		6250	8.70	0.58	0.67	7.45	0.98			0.98
" 17	900		525		5950	8.44	0.56	0.90	6.98	0.88			0.89
" 27	900		525		5700	8.65	0.65	1.21	6.79	1.00			0.98
July 10	900		500		5900	8.82	0.41	1.30	7.11	1.05			1.00
Horse 320													
Jan. 31	Normal				400			2.60	4.52	1.30	2.03	0.72	0.40
March 11	260	100	20		400	8.50	0.47	1.57	5.46	0.82	1.80	0.60	0.34
April 1	500	350	35	2	400	10.14	0.34	1.32	8.48	1.20	2.12	0.74	0.41
" 30	600	1050	50	50	400	9.63	0.74	1.22	7.67	0.45	2.61	0.90	0.50
June 8		1350	50	90	5600	8.55	0.49	1.35	6.71	0.40			
" 17		1100	30	100	7100	7.64	0.41	1.59	5.64	0.35	2.58	0.90	0.49
" 26	Diphtheria toxin discontinued	1100	10	90	6200	7.70	0.48	1.61	5.61	0.27	2.69	0.93	0.51

Tetanus toxin discontinued

in the contents of the several proteins) had been attained; routine bleedings of four to ten liters for antitoxin production were then instituted.

The two refractory, one medium and the eight horses yielding a highly potent antitoxic plasma, all showed an increase of from 40 to 114 per cent. in the total serumglobulin. For the refractory animals, this increase was 59.3 and 87.9 per cents. In one refractory and in one high horse, the serumglobulin maximum preceded the highest concentration in antitoxin. In seven of the horses, the greatest increase in the total serumglobulin was coincident with the maximum antitoxic potency. The serumglobulin increase, however, tended relatively to precede that of the antitoxin. In the two

[37] Analyses are given as grams of coagulable protein per 100 cubic centimeters of plasma.

other horses, both maxima also were observed together; no "intermediate" bleedings were made in these two instances. The greatest absolute increase in the serumglobulin was observed in the most potent plasma obtained in the series; the second place, however, went to a refractory horse. Subsequent to the maxima, the serumglobulin content was maintained at high concentration, in spite of repeated bleedings; it then only roughly paralleled the antitoxic variations in the plasma of the individual animals.

At dilutions of the plasma in the precipitated mixtures of 1:1.5, 1:5 and 1:10, the ammonium sulphate "euglobulin" fraction amounted to about 60 to 70, 20 to 24 and 10 to 15 per cents., respectively, of the total serumglobulin in both the normal and the antitoxic plasma. In an 850 unit plasma, an increase in the "euglobulin" over the normal percentage was observed, but the high content of protein in this plasma probably influenced the precipitation limits. The influence of the protein concentration is indicated by the different percentages for the "euglobulin" obtained for the three dilutions of 1:1.5, 1:5 and 1:10. The "euglobulin" then was not increased to a greater extent than the "pseudoglobulin" as the result of immunization, as has at times been maintained.

PROTEIN VARIATION DURING IMMUNIZATION.[38]

Horse 321.

Date of bleeding, 1907.	Last amount of diphtheria toxin injected.	Last amount of tetanus toxin injected.	Diphtheria units per cubic centimeter.	Tetanus units per cubic centimeter.	Amount of plasma recovered from bleeding.	At dilution 1:10; total protein.	At dilution 1:10; fibrinogen.	At dilution 1:10; serum albumin.	At dilution 1:10; serum globulin.	At dilution 1:10; sodium chloride insoluble globulin.	At dilution 1:1.5; euglobulin.	At dilution 1:5; euglobulin.	At dilution 1:10; euglobulin.
Feb. 6	Normal				400	—	—	2.31	4.53	1.66	2.69	1.03	0.59
March 11	200	75	60		400	9.51	0.49	2.16	7.86	1.20	5.17	1.88	1.00
April 1	400	300	150	2	400	10.20	0.46	1.14	8.60	1.03	5.73	2.06	1.20
Horse 322													
Feb. 21	Normal				400	—	—	2.10	3.93	0.92	2.70	0.88	0.50
March 25	110	70	50		400	7.70	0.48	1.53	5.69	1.34	3.80	1.31	0.71
April 30	450	600	625	5	400	8.77	0.51	0.77	7.49	0.46	5.20	2.27	1.34
May 3			600		4000	8.67	0.82	0.91	6.94	0.49	3.96	1.40	0.83
May 7			400		5000	8.04	1.00	0.55	6.49	0.49	3.24	1.17	0.64

[38] Analyses are given as grams of coagulable protein per 100 cubic centimeters of plasma.

PROTEIN VARIATION DURING IMMUNIZATION.[39]

Horse 323.

Date of bleeding, 1907.	Last amount of diphtheria toxin injected about seven days before date of bleeding.	Last amount tetanus toxin injected about seven days before date of bleeding.	Diphtheria units per cubic centimeter.	Tetanus units per cubic centimeter.	Amount of plasma recovered from bleeding.	At dilution 1:10; total protein.	At dilution 1:10; fibrinogen.	At dilution 1:10; serum-albumin.	At dilution 1:10; serum-globulin.	At dilution 1:10; sodium chloride insoluble globulin.	At dilution 1:1.5; euglobulin.	At dilution 1:5; euglobulin.	At dilution 1:10; euglobulin.
Feb. 26	Normal				400			2.33	4.60	1.16	2.21	0.68	0.37
March 25	110	70	50		400	8.79	0.74	1.98	6.07	2.06	4.06	1.45	0.82
April 29	400	525	850	5	400	10.49	0.71	0.78	9.00	0.66	4.50	1.53	0.85
May 10			550		6000	9.47	0.80	0.66	8.01	0.70	3.29	1.22	0.67
June 17	375		500		7700	8.29	0.39	1.45	6.45	0.46	2.85	1.00	0.57
July 2	475		500		8000	8.36	0.29	1.33	6.74	0.64			
" 10	500		450		6200	8.16	0.50	1.25	6.41	0.61	3.99	1.39	0.75
" 18	550		450		6200	7.92	0.32	1.14	6.46	0.79	3.80	1.30	0.71
" 29	600		450		6200	8.08	0.48	1.24	6.36	0.41			0.73
Aug. 9	650		425		6100	8.00	0.40	1.30	6.30	0.51			0.69
" 17	700		425		6200	8.20	0.47	1.32	6.41	0.58			0.68
" 27	750		425		6300	8.15	0.44	1.25	6.46	0.46			0.70
Sept. 3			425		6000	8.30	0.50	1.30	6.50	0.50			0.67
" 12	800		475		6250	8.35	0.55	1.33	6.47	0.47			0.68
" 19	800		500		5200	8.45	0.46	1.40	6.59	0.52			0.70
" 28	800		550		6400	8.30	0.50	1.55	6.25	0.50			0.65
Oct. 9	800		575		6100	8.50	0.45	1.75	6.20	0.55			0.55
" 19	800		550		6200	8.65	0.50	2.00	6.15	0.53			0.57
" 30	850		625		6200	8.71	0.53	2.18	6.00	0.48	2.87	0.99	0.55
Nov. 8	850		600		6100	9.10	0.53	2.00	6.57	0.64	2.92	1.01	0.55
" 19[40]	850		625		6300	8.56	0.51	1.73	6.32	0.57	3.15	1.12	0.61

Tetanus toxin discontinued

The " euglobulin " precipitated by saturating the plasma with sodium chloride (at ultimate dilution of the plasma 1:10) was much greater in normal plasma than the ammonium sulphate " eu-

[39] Analyses are given as grams of coagulable protein per 100 cubic centimeters of plasma.

[40] Subsequent bleedings were as follows: Nov. 27, '07, 600 units, 5,900 cubic centimeters; Dec. 6, '07, 600 units, 5,800 cubic centimeters; Dec. 16, '07, 600 units, 5,900 cubic centimeters; Dec. 27, 07, 500 units, 5,900 cubic centimeters; Jan. 6, '08, 400 units, 7,700 cubic centimeters; Jan. 14, '08, 350 units, 7,500 cubic centimeters; Jan. 22, '08, 400 units, 8,000 cubic centimeters; Jan. 31, '08, 400 units, 7,800 cubic centimeters; Feb. 7, '08, 425 units, 8,000 cubic centimeters; Feb. 15, '08, 425 units, 8,000 cubic centimeters; Feb. 24, '08, 475 units, 8,000 cubic centimeters; Mar. 4, '08, 300 units, 8,000 cubic centimeters; Mar 12, '08, 300 units, 8,000 cubic centimeters. There were no other bleedings. Over 200 liters of undiluted plasma were thus obtained from this one horse, representing the actual withdrawal of at least 275 liters of whole blood. The horse died suddenly on July 30, '08; toxin had not been given after Mar. 12, '08.

globulin" at the same dilution. There was a tendency in early immunization for this sodium chloride "euglobulin" to increase along with the total serumglobulin; it rapidly diminished, however, until at the height of immunization and maximum serumglobulin concentration, it may have reached less than half the normal absolute amount.

The serumalbumin was diminished a third to a half the normal along with the serumglobulin increase. Subsequent to the anti-

PROTEIN VARIATION DURING IMMUNIZATION.[41]

Horse 324.[42]

Date of bleeding, 1907.	Last amount of diphtheria toxin injected about seven days before date of bleeding.	Last amount of tetanus toxin injected about seven days before date of bleeding.	Diphtheria units per cubic centimeter.	Tetanus units per cubic centimeter.	Amount plasma recovered from bleeding.	At dilution 1:10; total protein.	At dilution 1:10; fibrinogen.	At dilution 1:10; serum-albumin.	At dilution 1:10; serum-globulin.	At dilution 1:10; sodium chloride insoluble globulin.	At dilution 1:5; euglobulin.	At dilution 1:5; euglobulin.	At dilution 1:10; euglobulin.
March 25	100	70	¼		400	7.88	0.45	1.88	5.55	0.98	3.72	1.37	0.74
April 30	400	525	25	1	400	10.43	0.76	0.83	8.84	0.63	3.30	1.18	0.65
May 31	725	Tet. toxin discontinued	125		400	9.49	0.61	0.64	8.24	0.56			0.84
June 19	900		125		8000	9.06	0.42	0.93	7.71	0.53	4.79	1.75	0.98
Horse 325													
Feb. 26	Normal				400			2.61	3.68	0.91	1.79	0.76	0.42
March 25	110	70	4		400	7.78	0.50	2.25	5.03	1.54	3.78	1.39	0.76
April 30	400	525	300	4	400	8.65	0.59	1.16	6.90	0.49	5.15	1.84	1.00
May 29		1000	200	25	400	7.74	0.54	1.41	5.79	0.50	4.72	1.74	0.98
July 2	550	Tetanus toxin discontinued	150		6800	8.11	0.39	1.63	6.09	0.90			1.00
" 10	600		150		5300	8.45	0.35	2.31	5.79	0.82			0.99
Sept. 3	1050		200		7300	7.95	0.40	2.10	5.45	0.90			0.97
" 17	1200		250		6100	8.10	0.41	2.30	5.39	0.84			0.95
" 28	1200		200		5350	7.90	0.36	2.29	5.25	0.80			0.93
Oct. 9	1200		200		5400	8.00	0.38	2.35	5.27	0.70			0.80
" 19	1300		175		5600	7.80	0.40	2.41	4.99	0 65			0.85
" 30	1300		150		1100	7.86	0.36	2.44	5.06	0.58	4.20	1.54	0.81
Nov. 8			125		400	7.61	0.30	2.55	4.76	0.64			0.67
" 19			75		400	6.95	0.55	2.29	4.11	0.37	2.37	0.81	0.52
Horse 326													
March 6	Normal				400	7.15	0.33	2.82	4.00	1.09	2.06	0.77	0.42
April 1	100	50	5		400	8.47	0.46	2.38	5.63	0.96	3.52	1.26	0.70
" 30	350	400	600	1	8000	9.41	0.64	1.16	7.61	0.42	4.68	1.66	0.90
May 10	400	Tet. toxin discontinued	600		8000	8.08	0.72	1.44	5.92	0.63	3.87	1.34	0.72
" 18	450		600		7800	8.73	0.79	0.98	6.96	0.61	3.78	1.29	0.70
" 29	500		600		8000	8.73	0.74	0.97	7.02	0.74	3.93	1.38	0.72
June 13	500		550		5600	10.52	0.68	0.61	9.23	1.01	6.46	2.38	1.30

[41] Analyses are given as grams of coagulable protein per 100 cubic centimeters of plasma.

[42] Immunization started Feb. 26, '07.

PROTEIN VARIATION DURING IMMUNIZATION.[43]

Horse 327.

Date of bleeding, 1907.	Last amount of diphtheria toxin injected.	Last amount of tetanus toxin injected.	Diphtheria units per cubic centimeter.	Tetanus units per cubic centimeter.	Amount of plasma recovered from bleeding.	At dilution 1:10; total protein.	At dilution 1:10; fibrinogen.	At dilution 1:10; serum albumin.	At dilution 1:10; serum globulin.	At dilution 1:10; sodium chloride insoluble globulin.	At dilution 1:1.5; euglobulin.	At dilution 1:5; euglobulin.	At dilution 1:10; euglobulin.
March 11	Normal				400	6.21	0.61	2.79	2.81	0.52	0.91	0.36	0.20
April 1	70	20	5		400	6.62	0.32	2.58	3.72	0.72	3.01	1.11	0.54
April 29	300	400	400	1	7300	8.03	0.66	1.69	5.68	0.80	4.13	1.41	0.75
Horse 328													
March 11	Normal				400	6.53	0.50	2.52	3.51	0.91	1.61	0.59	0.31
April 1	70	20	4		400	7.60	0.26	2.12	5.22	0.92			
April 27	300	400	550	1	4000	9.39	0.75	1.13	7.51	0.77	4.73	1.69	0.94

toxic and serumglobulin maxima, figures as low as a fifth of the original serumalbumin content have been noted.

It is suggested that this diminution of the sodium chloride "euglobulin" and the serumalbumin is a physiological compensation for the greater viscosity of the plasma because of the increase in the more soluble serumglobulins.

No characteristic alteration in the fibrinogen of the plasma was observed during immunization. Individual variations up to 0.5 gram per 100 cubic centimeters of plasma have been recorded.

The influence of repeated bleedings does not essentially influence the protein changes induced by immunization. These remarkable regenerative processes are worthy of note.

The results of our investigation indicate that in "forced" immunization, the same characteristic quantitative changes can occur in the blood proteins of both refractory horses and those yielding a highly potent antitoxic plasma. We cannot conclude, however, that the serumglobulin increase does not represent an accumulation of antitoxin, at least in part; it is possible that other antibodies may be formed either prior to or along with the specific antitoxin and that these may constitute a portion of the increase in the more soluble serumglobulin with which protein such substances are associated.

[43] Analyses are given as grams of coagulable protein per 100 cubic centimeters of plasma.